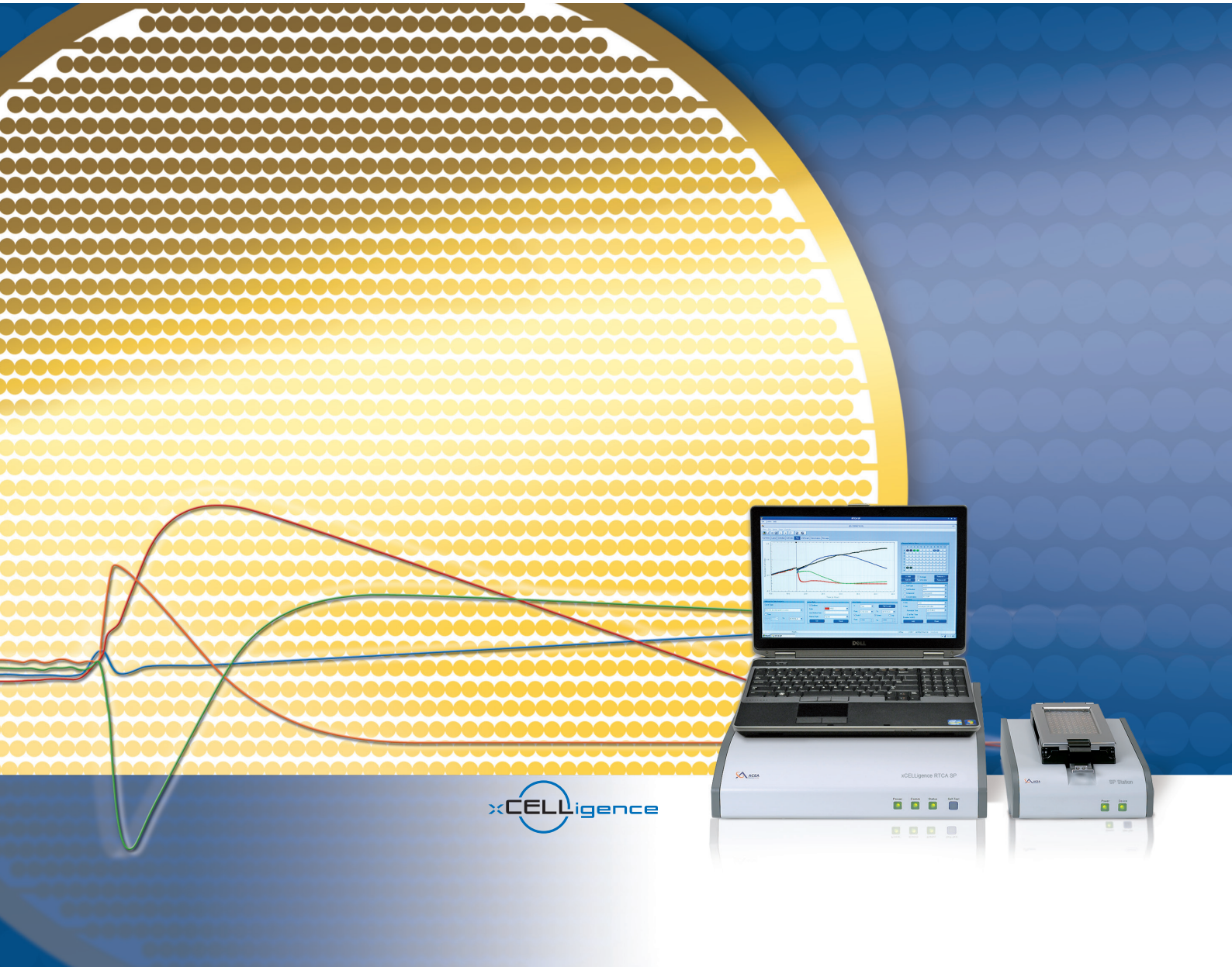
xCELLigence® RTCA SP Operator's Manual

Version December 2018



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Prologue

I. Revision History

Version	Revision Date
1.0	June 2008
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1.0 (Document Number Update)	November 2015
2.0	December 2018

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Questions or comments regarding the contents of this manual can be directed to the address below or to your local ACEA representative.

ACEA Biosciences, Inc.
Customer Support
6779 Mesa Ridge Road, Suite 100
San Diego, CA 92121
USA

Every effort has been made to ensure that all the information contained in the xCELLigence RTCA SP Operator's Manual is correct at the time of printing.

However, ACEA Biosciences, Inc. reserves the right to make any changes necessary without notice as part of ongoing product development.

II. Contact Information

	Manufacturer	ACEA Biosciences, Inc. 6779 Mesa Ridge Road, Suite 100 San Diego, CA 92121 USA
	China Distributor	ACEA Bio (Hangzhou) Co., Ltd. Building 5, 2# Xiyuan Wu Road, Xihu District Hangzhou, Zhejiang Province, P. R. China 310030
	International Distributors	Please contact ACEA Biosciences, Inc., or visit www.aceabio.com for a complete list of international distributors

III. Declaration of Conformity

CE	The instrument meets the requirements laid down in Council Directive 2014/30/EU relating to "Electromagnetic Compatibility" and Council Directive 2014/35/EU relating to "Low Voltage Equipment". The following standards were applied: IEC/EN 61326 (EMC) and IEC/EN 61010-1 (Safety).
SGS C US	The instrument meets the requirements laid down in UL 61010-1 and CAN/CSA-C22.2 No. 61010-1-12 Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use – Part 1: General Requirements.

IV. Warranty

Information on warranty conditions are specified in the sales contract. Contact your ACEA representative for further information.

Any unauthorized modification of the instrument invalidates the guarantee and service contract.

V. Trademarks

XCELLIGENCE, E-PLATE and ACEA BIOSCIENCES are registered trademarks of ACEA Biosciences, Inc. in the US.

Other brands or product names are trademarks of their respective holders.

VI. Intended Use

The xCELLigence RTCA (Real-Time Cell Analyzer) SP (Single Plate) is intended for label-free, real-time, automated monitoring of cell status in a variety of cell-based assays, using proprietary micro-electronic sensor technology developed by ACEA Biosciences. It can be used for both high throughput screening and research laboratory environments.

The xCELLigence RTCA SP is intended **for life science research** and must be used exclusively by laboratory professionals who are trained in laboratory techniques and have studied the instructions for use of this instrument. **The xCELLigence RTCA SP is not intended for use in diagnostic procedures.**

VII. License Statements for the Instrument

This xCELLigence RTCA SP is a real-time cell based assay system covered by US patent No.7,192,752, 7,459,303, 7,468,255, 7,470,533, 7,560,269, 7,732,127, 7,867,108, 8,026,080, 8,041,505; European patent applications 03748948.1, 03751801.6 and 04801001.1, 05722991.6, 05786773.1, and other patents and patent applications owned by ACEA Biosciences, Inc..

VIII. Software License Agreement

An envelope containing all license and end user agreements is packaged with the xCELLigence RTCA SP/MP/DP Control Unit. It contains:

- RTCA Software End User License Agreement
- End User License Agreement for Microsoft Windows 7 Professional
- End User License Agreement for Microsoft Office Home & Business
- Microsoft Office Home & Business Product Key

Please comply with all statements made in these documents.

IX. Preamble

The xCELLigence RTCA SP Operator's Manual has to be used in conjunction with the corresponding RTCA Software Manual. Before setting-up operation of the xCELLigence RTCA SP, it is important to read this Operator's Manual and the corresponding RTCA Software Manual thoroughly and completely. Failure to observe the instructions contained in this manual could be hazardous.

X. Use of the Instrument Operator's Manual

This Operator's Manual describes the operation of the xCELLigence RTCA SP and contains the following chapters:

Chapter A Overview contains a short introduction to the operation of the xCELLigence RTCA SP and describes the instrument's specifications.

Chapter B System Description contains the description of the xCELLigence RTCA SP, and the instructions on the installation of the instrument.

Chapter C Operation describes the operating procedures for the xCELLigence RTCA SP.

Chapter D Maintenance and Care describes the maintenance procedures that are required for the xCELLigence RTCA SP.

Chapter E Appendix contains trouble shooting and ordering information of the xCELLigence RTCA SP, accessories and consumables.

XI. Conventions Used in this Manual

Text Conventions

To impart information that is consistent and memorable, the following text conventions are used in this Operator's Manual:

Numbered Listing	Describes the steps in a procedure that must be performed in the order listed.
Italic type, gold	Points to a different chapter in this Operator's Manual which should be referred to for better understanding.
Italic type	Describes buttons, icons or functions when operating the RTCA Software. In addition, important notes and information notes are shown in italic type.

Symbols

In this Operator's Manual symbols are used as a visual signal to highlight important points.

Symbol	Heading	Description
	WARNING	This symbol is used to indicate that noncompliance with instructions or procedures could lead to physical injury or even death or could cause damage to the instrument. Consult this Operator's Manual.
	BIOHAZARD	This symbol indicates that certain precautions must be taken when working with potentially infectious samples material.
	IMPORTANT NOTE	Information critical to the success of the procedure or use of the product.
	INFORMATION NOTE	Additional information about the current topic or procedure.
▶▶▶		Table continued on next page.
■		End of table.

The following symbols appear on the instrument:

Symbol	Heading	Description
	AC	Alternating Current
	ON/OFF	Power ON Power OFF
	GND	Protective Earth Ground
	FUSE	Fuse: Refer to included safety statements
	WEEE	Electrical and electronic equipment marked with this symbol are covered by the European Directive WEEE. The symbol denotes that the equipment must not be disposed of in the municipal waste system.
	BIOHAZARD	This symbol indicates that certain precautions must be taken when working with potentially infectious material.
COM	COM	Communications Port
EPS	EPS	E-Plate Station Port

XII. Warnings and Precautions

Handling Requirements

The xCELLigence RTCA SP must only be used by trained and skilled personnel. It is essential that the following safety information required for installation and operation of the xCELLigence RTCA SP is carefully read and observed. Please ensure that this safety information is accessible to every employee working with the xCELLigence RTCA SP.

- Follow all safety instructions printed on or attached to the instrument.
- Observe all general safety precautions applicable to electrical instruments.
- Do not access any electrical parts while the xCELLigence RTCA SP is connected to the mains.
- Never touch switches or power cords with wet hands.
- Do not open the housing of the xCELLigence RTCA SP.
- Never clean the instrument without turning the instrument power switch off and disconnecting the power cord.
- Only authorized service personnel are allowed to perform service operations or repairs required for this instrument.
- Do not open the clamp plate of the SP Station during measurement as this will cause an open circuit, leading to an error message in the RTCA Software.



For your own safety, please consider all biological material as potentially infectious. Handling and disposal of such material should be performed according to local safety guidelines. Spills should be immediately disinfected with an appropriate disinfectant solution to avoid spreading infection to, and contamination of, laboratory personnel or equipment. When working with potentially infectious material, please do always use protective gloves (powder-free).

- Please refer to the section on *Maintenance and Care* for instructions on cleaning the xCELLigence RTCA SP.

General Precautions

	Microsoft Office and AVG AntiVirus FREE software have been tested and do not interfere with the RTCA Software. No other additional software may be installed on the xCELLigence RTCA SP/MP/DP Control Unit. Installation of any other additional software on the xCELLigence RTCA SP/MP/DP Control Unit involves the risk of interference with RTCA Software, and could affect the result or control unit security.
	Make sure that the main switch is freely accessible. Inappropriate location can cause incorrect results and damage to the equipment parts. Follow the installation instructions carefully. Keep all potentially inflammable or explosive material (for example, anesthetic gas) away from the instrument. Spraying liquid on electrical parts can cause a short circuit and result in a fire.
	Keep the cover closed while the instrument is connected to the mains and do not use sprays in the vicinity of the xCELLigence RTCA SP. During firefighting operations, disconnect the xCELLigence RTCA SP from the mains.
	Do not use the instrument where liquids or chemicals are used or gas may be produced. Insure that no liquids are spilled into the instrument. If there is any spillage disconnect mains immediately and return to product for serving.

Electrical Safety

	The xCELLigence RTCA SP is designed in accordance with Protection Class I (IEC). The chassis/housing of the instrument is connected to protection earth (PE) by means of a cord. For protection against electrical shock hazards, the instrument must be directly connected to an approved power source such as a three-wire grounded receptacle for the 110 V or 230 V line. Where an ungrounded receptacle is encountered, a qualified electrician must replace it with a properly (PE) grounded receptacle in accordance with the local electrical code. An extension must not be used. Any break in the electrical ground path, whether inside or outside the instrument, could create a hazardous condition. Under no circumstances should the user attempt to modify or deliberately defeat the safety features of this instrument. If the power cord becomes cracked, frayed, broken, or otherwise damaged, please contact ACEA Biosciences. Do not replace with a lower rated cord. If the equipment is used in a manner not specified by ACEA Biosciences, the protection provide by the equipment may be impaired.

XIII. Disposal of the Instrument

All electrical and electronic products should be disposed of separately from the municipal waste system. Proper disposal of your old appliance prevents potential negative consequences for the environment and human health.



The instrument must be treated as biologically contaminated-hazardous waste. Final disposal must be organised in a way that does not endanger waste handlers. As a rule, such equipment must be sterile before it is passed on for final disposal.

For more information contact your local ACEA Support personnel.

The instrument should also be decontaminated prior to shipping for outside service or repairs.



Components of your Control Unit such as the computer, monitor, keyboard, etc. which are marked with the crossed-out wheeled bin symbol are covered by the European Directive 2002/96/EC (WEEE).

These items must be disposed of via designated collection facilities appointed by government or local authorities.

For more information about disposal of your old product, please contact your city office, waste disposal service or your local ACEA Support personnel.



It is left to the discretion of the responsible laboratory organization to determine whether the xCELLigence RTCA SP/MP/DP Control Unit and xCELLigence RTCA SP/MP Analyzer are contaminated or not. If contaminated, treat in the same way as the SP Station.

A Overview

1. Introduction

Over 95% of current biological assays are based on optical detection (*e.g.*, fluorescent and optical detection in a fluorescence plate reader, optical microscopy, FACS analyzer, fluorescence scanner). In contrast, the new xCELLigence RTCA SP developed by ACEA Biosciences is based on electronic detection of biological assay processes, thereby integrating molecular and cell biology with microelectronics.

The presence, absence, or change in properties of cells or molecules affects the passage of electrons and ions on sensor surfaces. Measuring the electronic properties of sensor surfaces provides important information about the biological status of cells (or proteins) present on the sensors. When changes occur in the biological status of cells or proteins, the xCELLigence RTCA SP automatically measures the corresponding changes in the electronic properties of the sensors near the cells. These analog electronic readout signals are then converted to digital signals for processing and analysis. The major advantages of the xCELLigence RTCA SP for biological assays over conventional approaches include:

- Label-free detection
- Non-invasive, real-time monitoring
- Automated measurement
- Easy set-up, operation and control
- High information content
- High sensitivity and accuracy
- Broad range of applications

This new microelectronic sensing system represents one of the first integrations of electronic methodology and cell-based assays.

A

1.1 xCELLigence RTCA SP

The new xCELLigence RTCA SP (Single Plate) is a proprietary microelectronic biosensor system for cell-based assays. The core of the system is the microelectronic cell sensor arrays that are integrated into the bottom of 96-well plates (E-Plate 96). Measuring the electronic impedance of these sensor electrodes allows changes in cells on the electrodes to be detected and monitored. Cell viability, cell number, cell morphology, and degree of adhesion all affect electrode impedance.

Impedance measured between electrodes in an individual well depends on electrode geometry, ionic concentration in the well and whether cells are attached to the electrodes. In the absence of cells, electrode impedance depends mainly on the ionic environment both at the electrode/solution interface and in the bulk solution. In the presence of cells, cells attached to the electrode sensor surfaces will act as insulators and thereby alter the local ionic environment at the electrode/solution interface, leading to an increase in impedance. Thus, the more the cells there are on the electrodes, the larger the change in electrode impedance (Figure 1).

Furthermore, the impedance change also depends on the extent to which cells attach to the electrodes (Figure 1). For example, cell spreading resulting in a large cell/electrode contact area leads to a large change in impedance.

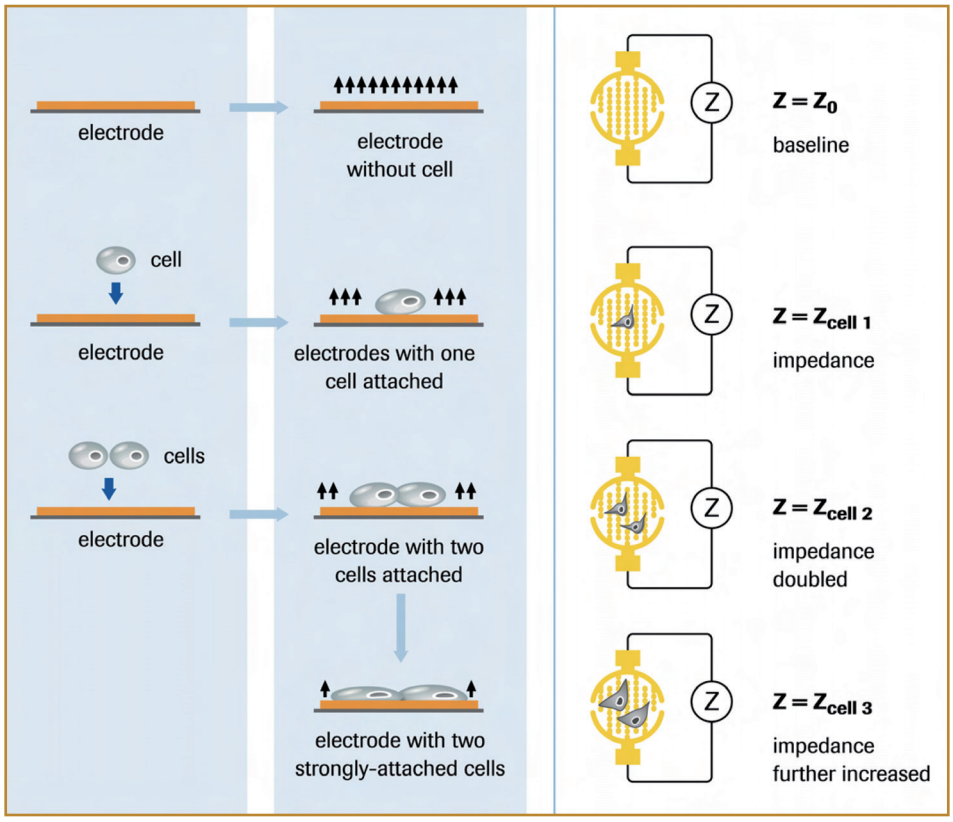


Figure 1: Schematic illustration of the impedance measurement principle.

Derivation of the Cell Index (CI)

A unitless parameter termed Cell Index (CI) is derived to measure the relative change in electrical impedance to represent cell status. The CI is a relative and dimensionless value since it represents the impedance change divided by a background value. When there are no cells present in the medium, the sensor's electronic property will not be affected and the impedance will be small as shown in Figure 1 when $Z = Z_0$.

When there are more cells on the electrodes, the impedance will be larger. The CI calculation is based on the following formula: $CI = (Z_i - Z_0)/15\ \Omega$, where Z_i is the impedance at an individual point of time during the experiment, and Z_0 is the impedance at the start of the experiment. Thus the CI is a self-calibrated value derived from the ratio of impedances. For more detailed information on how to derive the CI, please refer to the *Monitor the Experiment* section in the RTCA Software Manual. Several features of the CI are summarized:

- When cells are not present or are not well-adhered on the electrodes, then the CI is about zero.
- Under the same physiological conditions, when more cells are attached on the electrodes, then the CI values are higher. In this case, CI is a quantitative measure of the cell number present in a well.
- Additionally, a change in a cell status, such as cell morphology, cell adhesion or cell viability can lead to a change in CI.

The measurement of impedance is a non-invasive method in relation to the cells, and only a very weak electrical signal is applied to the sensor electrodes. The AC excitation voltage level is in the mV range and the resulting current is in the µA range so that individual wells in a plate can be interrogated repeatedly over a wide interval range throughout the experiment. Chemical compounds or other reagents can be added at any time and monitored continuously in hours or days. Unlike "end point" assays, the system allows an investigator to monitor the entire course of the experiment in real time without any interruption.

The xCELLigence RTCA SP consists of the following main components:

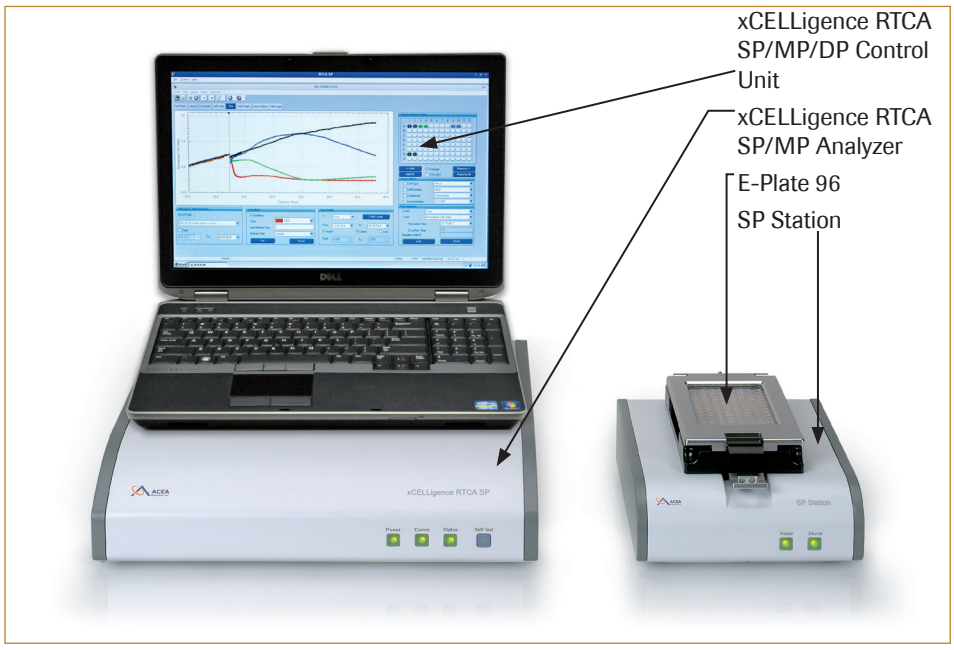


Figure 2: The xCELLigence RTCA SP with the main components: xCELLigence RTCA SP/MP Analyzer, SP Station, E-Plate 96 and xCELLigence RTCA SP/MP/DP Control Unit.



Cells are introduced into the wells of the E-Plate 96, making contact with, and attaching to the sensor-electrode surfaces. The electronic properties of the sensor surfaces are monitored through the SP Station, which can be placed inside a tissue culture incubator, and the xCELLigence RTCA SP/MP Analyzer. This is done under the control of the RTCA Software on the xCELLigence RTCA SP/MP/DP Control Unit, to provide real-time, quantitative information about the biological status of the cells, including cell number, viability, morphology and cytoskeletal dynamics.

The RTCA Instrument is applicable for a wide variety of cell-based assay applications, including:

- Cell adhesion and spreading
- Cell proliferation and differentiation
- Compound-mediated cytotoxicity/Apoptosis
- Cell-mediated cytotoxicity
- Receptor-mediated signaling
- Quality control of cells
- Virus-mediated cytopathogenicity

The System is highly quantitative, with excellent accuracy, precision, and ease of use. The RTCA Software can be used for simplified experimental design and set-up; it will then direct the instrument to perform the experiment and provide fully automated data acquisition, data analysis, and data presentation.

A

2. Specifications of the Instrument

The xCELLigence RTCA SP has to be operated at an altitude/pressure of 0 – 2000 m above sea level, 80 – 106 kPa.

2.1 Specifications of the xCELLigence RTCA SP/MP Analyzer

- Model: W380
- Dimensions: 40.0 cm × 40.0 cm × 9.0 cm (W × D × H)
- Weight: 7.4 kg
- Electrical Input: 100 – 240 VAC, 50/60 Hz, 25 W max
- Output test signal: 22 mV rms ± 20% with max. 5 mV DC offset at 10, 25 and 50 kHz
- Impedance Measurement Accuracy: ± (1.5% + 1 Ω)
- Impedance Measurement Repeatability: 0.8%
- Impedance Dynamic Range: 10 Ω to 5 kΩ
- Communication: RS232 Serial communications at a baud rate of 57600 bits/second
- Environment: indoor use; temperature, +15°C to +32°C; relative humidity, 80% max. up to +32°C, without condensation

2.2 Specifications of the SP Station

- Dimension: 21.0 cm × 25.7 cm × 10.7 cm (W × D × H)
- Weight: 3.6 kg
- Electrical Input: +5 V, -5 V, 5 W max
- Electronic Switch Resistance: 2 – 5 Ω
- Electrical Interface: Handling one E-Plate 96
- Communication: RS232 Serial communications at a baud rate of 57,600 bits/second
- Environment: temperature, +15°C to +40°C; relative humidity, 98% maximum without condensation

2.3 Specifications of the xCELLigence RTCA SP/MP/DP Control Unit

- ≥ 160 GB Hard disk drive
- ≥ 2 GB RAM
- ≥ 256 MB Graphics device

The Windows® Operating System (OS) delivered with the xCELLigence RTCA SP/MP/DP Control Unit is modified to ensure optimal performance and operation with the xCELLigence RTCA SP. These modifications restrict certain network capabilities such as sharing folders and printers to the network. However, to facilitate the transport of data to and from the xCELLigence RTCA SP/MP/DP Control Unit, the following network features remain enabled:

- Mount Network Drives for data exchange
- FTP (File Transfer Protocol)
- Automatically update Virus Definition Files

A

2.4 Specifications of the E-Plate 96

- Footprint: complying with ANSI/SBS 1-2004 requirements
- Dimension: 12.77 cm × 8.55 cm × 1.75 cm (W × D × H) (with plate cover)
- Spacing: The spacing of the wells is 9 mm center-to-center as per the ANSI/SBS 4-2004 standard for 96-well titer plates
- Volume: 243 µl ± 5 µl
- Bottom Diameter: 5.0 mm ± 0.05 mm
- Electrical Interface: Interface with SP Station and MP Station
- Sensor Impedance: 17 Ω ± 5 Ω at 10 kHz, when measured with a 1X PBS Solution
- Material: Biocompatible surfaces
- UV irradiated
- Environment: temperature, +15°C to +40°C; relative humidity, 98% maximum without condensation

2.5 Specifications of the RTCA Resistor Plate 96

- Dimension: 12.77 cm × 8.55 cm × 1.75 cm (W × D × H) (with plate cover)
- Electrical Interface: Interface with SP Station and MP Station
- Environment: temperature, +15°C to +40°C; relative humidity, 98% maximum without condensation
- Resistor values: 37.4, 64.9, 91.0, 115.0 Ω, ± 0.5%



The resistor values specified for the RTCA Resistor Plate 96 are different variables than raw scan data acquired during resistor plate verification. Therefore, they are not comparable to acceptance values listed in chapter Resistor Plate Verification of the xCELLigence RTCA SP.

B System Description

1. System Package

The table below lists all the components required for the xCELLigence RTCA SP. Check for completeness and inspect packaging prior to installation.

The original shipping boxes must be transferred unopened to the installation site. On delivery, carefully inspect the boxes. It is essential that you report any suspected damage immediately to your local ACEA representative and to the shipping agent before accepting the product.

Use only the original packaging for transportation or relocation of the equipment.

Quantity	Component
1	**xCELLigence RTCA SP/MP Analyzer (SP)**
1	Power Cables for xCELLigence RTCA SP/MP Analyzer
1	Cable Connection between xCELLigence RTCA SP/MP Analyzer and xCELLigence RTCA SP/MP/DP Control Unit (serial cable)
1	USB-Serial Converter Cable
1	**SP Station**
1	Cable Connection between xCELLigence RTCA SP/MP Analyzer and SP Station (flat, shielded cable)
1	RTCA Protector Shield 96
1	RTCA Resistor Plate 96
1	RTCA Frame 96
10	RTCA Contact Pins 96
1	Pliers
1	RTCA Cleaning Kit, including Brush and Dust Blower
1	RTCA Contact Pin Insertion Tool
1	Tweezers
1	**xCELLigence RTCA SP/MP/DP Control Unit** (RTCA software pre-installed)
2	RTCA Software, Single License
1	Power Adapter and Cables for xCELLigence RTCA SP/MP/DP Control Unit

B

2. System Description

The xCELLigence RTCA SP is designed for label-free cell based assays. The instrument integrates microelectronics with cell and molecular biology. The core of the instrument is a microelectronic biosensor array incorporated into each well of standard-sized 96-well microplate devices. This permits the xCELLigence RTCA SP to measure the activity of living cells in real-time without any labels or reporters. Any number of cellular parameters such as attachment, spreading, growth, death, and even specific morphological changes can be simply and reliably detected with the xCELLigence RTCA SP.

The xCELLigence RTCA SP includes the following main components:

- xCELLigence RTCA SP/MP Analyzer
- SP Station
- xCELLigence RTCA SP/MP/DP Control Unit with RTCA Software preinstalled
- E-Plate 96

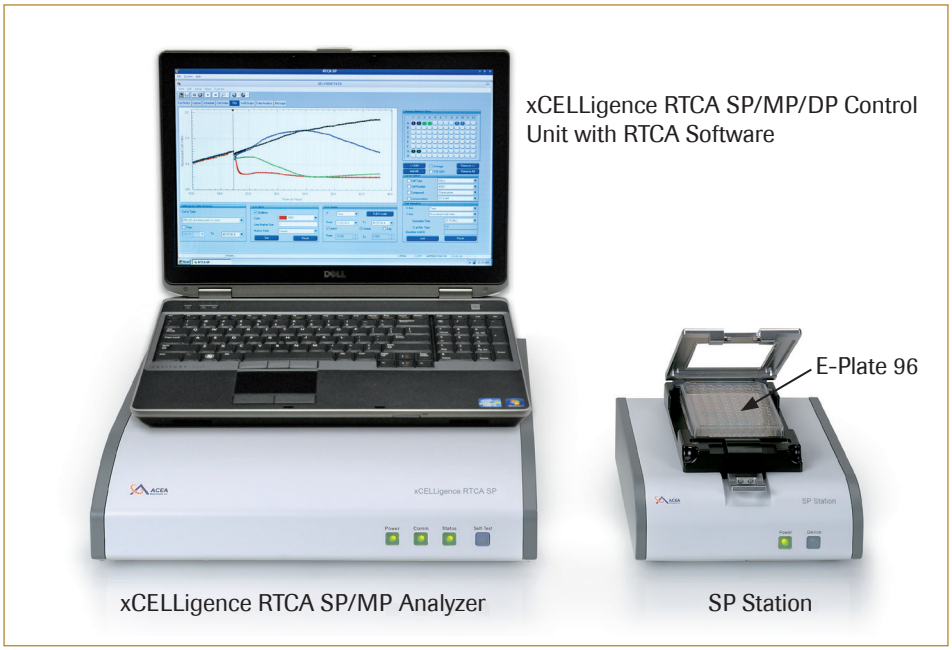


Figure 3: The xCELLigence RTCA SP.

In addition, several accessories are required to use the xCELLigence RTCA SP:

- RTCA Protector Shield 96
- RTCA Frame 96
- RTCA Resistor Plate 96
- RTCA Contact Pins 96
- RTCA Cleaning Kit (Brush + Dust Blower)
- RTCA Contact Pin Replacement Tool, Insertion Tool and Tweezers
- Connective and Power Cables

B

2.1 Description of the xCELLigence RTCA SP/MP Analyzer

The xCELLigence RTCA SP/MP Analyzer (in short "SP/MP Analyzer") is an electronic analyzer that can, under the control of RTCA Software, conduct electronic impedance measurement of sensor electrodes at various signal frequencies. The SP/MP Analyzer is capable of computer-controlled signal generation, processing and analysis, automatic frequency scanning and rapid measurement. The average measurement speed is approx. 15 seconds for a 96 well plate, or approx. 150 ms for each well.



Figure 4: The SP/MP Analyzer.

Interfaces and indicators:

The SP/MP Analyzer communicates with the xCELLigence RTCA SP/MP/DP Control Unit through a standard 9-pin RS-232 serial port designated as COM. Signals and communications to the SP Station are through the EPS port, both of these two ports are located at the rear panel of the SP/MP Analyzer.



Figure 5: The SP/MP Analyzer rear panel.

B

Keypad and indicators:

- Power LED, solid green if power is on.
- Communication LED, blinks if communication between SP Station and SP/MP Analyzer is active.
- Status LED, indicates system status. Solid green if power is on, blinks green if measurement is in progress, also blinks green once if self-test is passed. Red indicates a system error, such as failed self-test, no station or communication error.
- Self-test button, the status LED will flash green once if test is passed. It is RED if self-test is failed.

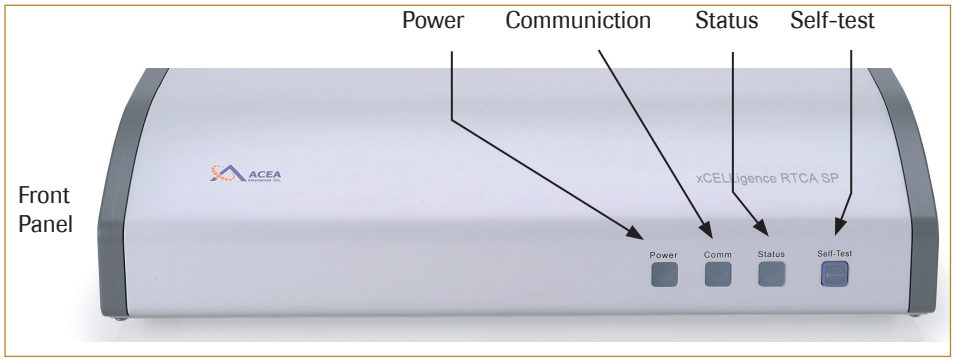


Figure 6: The SP/MP Analyzer front panel.

xCELLigence RTCA SP/MP Analyzer (SP)	Cat. No. 00380601510

2.2 Description of the SP Station

The SP Station is placed inside the incubator and used to connect E-Plates 96 to the SP/MP Analyzer. Appropriate connection and precision electronic switches in the SP Station enable software control or selection of individual electrode sensors within an E-Plate 96 to be measured by the SP/MP Analyzer.

The cradle pocket of the SP Station contains 120 individual, spring-loaded RTCA Contact Pins 96, each for connecting to one of the 120 conductive pads on the back of an E-Plate 96.

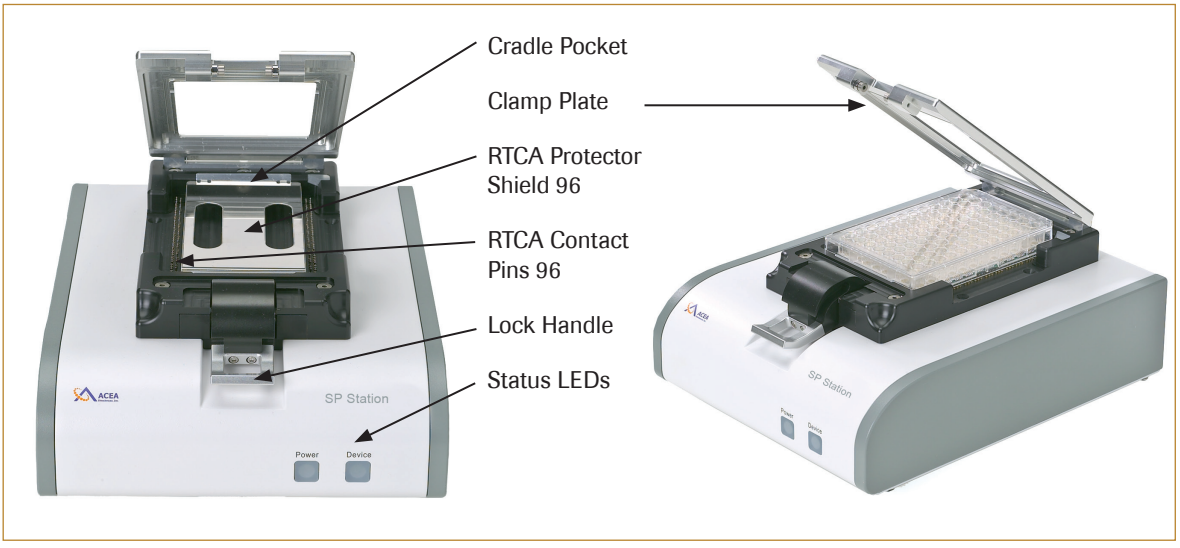


Figure 7: The SP Station.

2.2.1 Cradle Pocket and Clamp Plate

The cradle pocket and the clamp plate of the SP Station provide mechanical support and electrical connection from the E-Plates 96 to the electronic circuits of the station. The cradle pocket assembly connects the sensors in each well of the E-Plate 96 to the PCB board through the RTCA Contact Pins 96.

2.2.2 RTCA Protector Shield 96 and RTCA Contact Pins 96

In electronic equipment, a Contact Pin is a device that provides a frequent but temporary electrical connection. It is widely used in ATEs (automatic test equipment) for circuit testing. In the SP Station, the RTCA Contact Pins 96 are very important for ensuring electrical connection between the sensor and the control board.
Since it is very important to keep the RTCA Contact Pins 96 from being bent or damaged, an RTCA Protector Shield 96 ensures that the pins are not damaged during insertion or removal of the E-Plate 96.

To ensure good electrical connection, the RTCA Contact Pins 96 need to be cleaned regularly. Thus, it is recommended to clean the RTCA Contact Pins 96 every three months. More frequent cleaning may also be necessary, such as when certain positions show larger contact resistance.

The RTCA Contact Pins can also be replaced. Please refer to the *Maintenance and Care* section of this manual for instructions on the cleaning and replacement of RTCA Contact Pins 96.

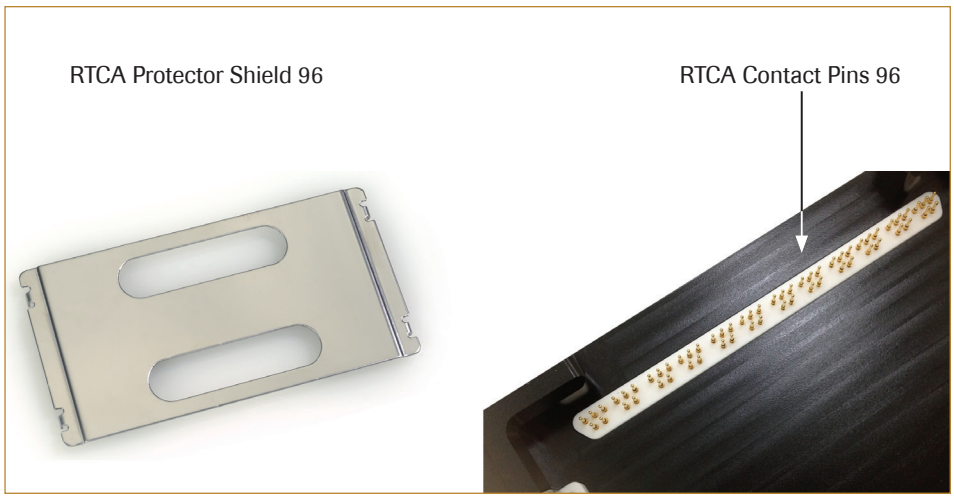


Figure 8: RTCA Contact Pins 96 and RTCA Protector Shield 96.

SP Station	Cat. No. 05229057001

B

2.3 xCELLigence RTCA SP/MP/DP Control Unit

The xCELLigence RTCA SP/MP/DP Control Unit consists of a laptop computer (Figure 9). The operating system and all software tools necessary to run the xCELLigence RTCA SP are already pre-installed.



Figure 9: The xCELLigence RTCA SP/MP/DP Control Unit.

xCELLigence RTCA SP/MP/DP Control Unit	**Cat. No. 05454417001**

2.4 E-Plate 96 and RTCA Resistor Plate 96

E-Plates 96 are single use, disposable devices used for performing cell-based assays on the xCELLigence RTCA SP. The E-Plate 96 is similar to commonly used 96x well micro-titer plates. Each well of the 96 wells on the E-Plates 96 has incorporated sensor electrode arrays so that cells inside each well can be monitored and assayed.

The plate has a low evaporation lid design. The bottom diameter of each well is 5.0 mm ± 0.05 mm, with a total volume of 243 µl ± 5 µl. Approx. 80% of the bottom area of each well are covered by the electrodes. The plate is designed to be used in an environment of +15°C to +40°C, relative humidity 98% maximum without condensation.

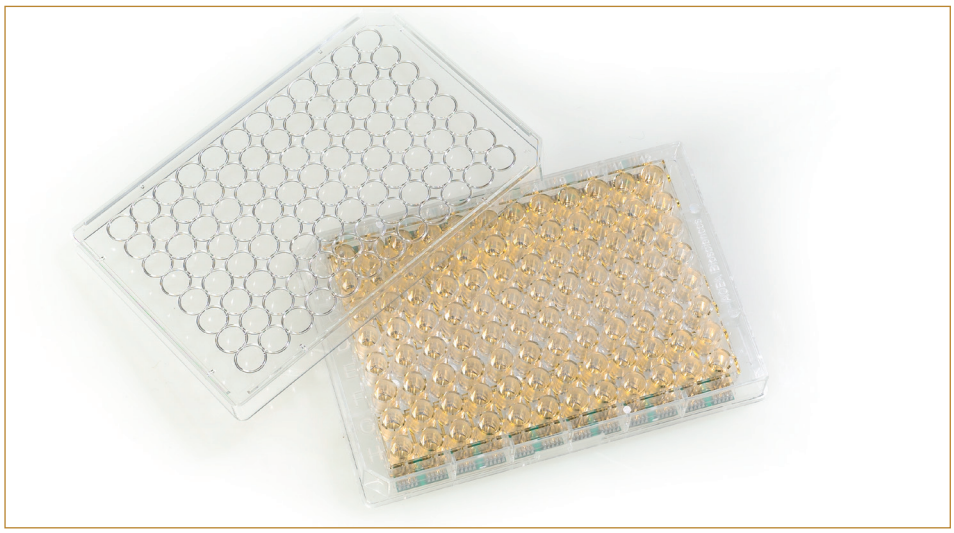


Figure 10: The E-Plate 96.

For system verification purpose, the RTCA Resistor Plate 96 is used with the xCELLigence RTCA SP as a standard accessory (see section on *Resistor Plate Verification of the xCELLigence RTCA SP*). The RTCA Resistor Plate 96 has the same size as the E-Plate 96. Instead of a micro sensor array, an array of resistors is placed at each well position in such a way that it shows resistance values close to that of an E-Plate 96 when only cell media are put into each well.

System functionality can be verified by putting the RTCA Resistor Plate 96 in the SP Station.

RTCA Resistor Plates 96 are not instrument-specific. Each RTCA Resistor Plates 96 can be used to perform Resistor Plate Verification on each SP Station or RTCA MP Station.

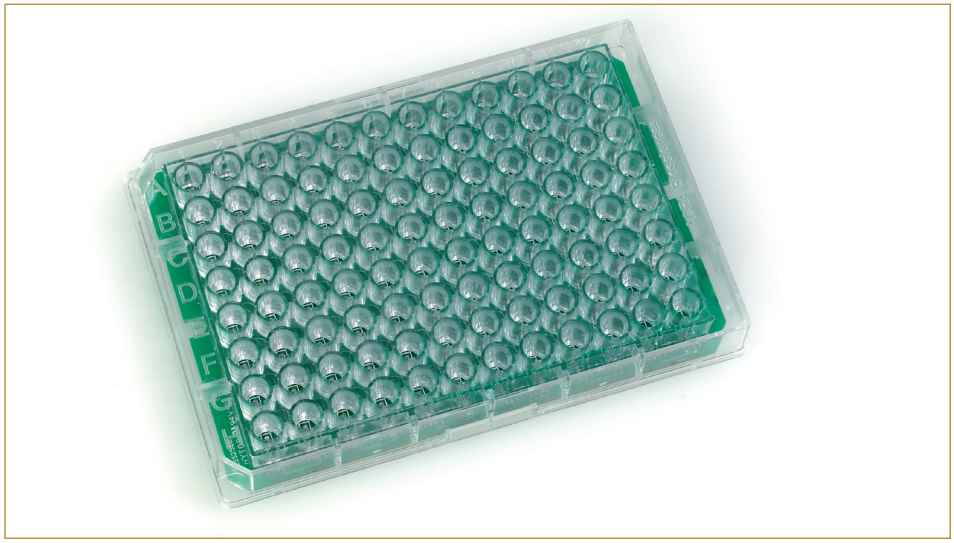


Figure 11: The RTCA Resistor Plate 96.

B

2.5 RTCA Frame 96

For experiments in which an extremely short reaction time is expected between the addition of a substance and the reaction of the cells, the addition of compounds directly into the wells without removal of the E-Plate 96 from the SP Station can prove highly advantageous. To allow for such an experimental setup, the RTCA Frame 96 can be used as a substitute for the usual lid of the E-Plate 96.

With the RTCA Frame 96 in place, you can directly pipette into the wells while the E-Plate 96 is locked in the SP Station.



Figure 12: The RTCA Frame 96.

RTCA Protector Shield 96		**Cat. No. 05288967001**
RTCA Resistor Plate 96		**Cat. No. 05232350001**
RTCA Frame 96		**Cat. No. 05232392001**
E-Plate 96	6 Units	**Cat. No. 05232368001**
	6 x 6 Units	**Cat. No. 05232376001**
RTCA Contact Pins 96 (20 Units)		**Cat. No. 05232384001**

2.6 RTCA Software

The RTCA Software provides unparalleled instrument control for flexible experiment setups, data acquisition, and data analysis. All instrument and experiment controls are embedded in the RTCA Software to simplify instrument setup and operation. The Software is used with the SP/MP Analyzer in conjunction with a single SP Station.

For a comprehensive description of the RTCA Software please refer to the RTCA Software Manual.

RTCA Software Package	Cat. No. 05454433001

B

2.7 RTCA Cleaning and Replacement Equipment

Tools for replacing and inserting the RTCA Contact Pins

The Pliers, Tweezers and RTCA Contact Pin Insertion Tool are used to remove defective pins from the SP Station and to insert new Contact Pins. The Contact Pins can be extracted using pliers and then be safely inserted using tweezers and the RTCA Contact Pin Insertion Tool.



Figure 13: The Pliers (Upper part), Tweezers and RTCA Contact Pin Insertion Tool (lower part).

RTCA Cleaning Kit

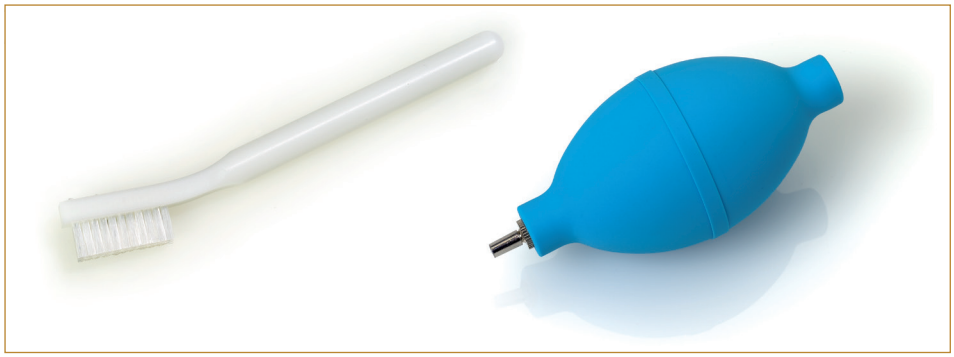


Figure 14: The RTCA Cleaning Kit.

3. Installation

3.1 Installation Warnings

Always make sure that the power switch on the SP/MP Analyzer is in the OFF position, and remove the power cord from the back of the instrument before installing or relocating the instrument.

Do not operate the instrument in an environment where potentially damaging liquids or gases are present.

Please do NOT put the xCELLigence RTCA SP/MP/DP Control Unit and SP/MP Analyzer in the incubator!

Connect the SP/MP Analyzer to a grounded AC supply line using the supplied cord. This is not only for safety purposes, but also for better noise performance of the xCELLigence RTCA SP. While a stand-alone xCELLigence RTCA SP can work with a two-pronged AC power source, but within a complicated electrical environment, it is highly advisable to use a well-grounded power socket, otherwise there may be a risk of electrical shock, and/or the performance of the xCELLigence RTCA SP may be affected.

*After installing an SP Station in an incubator, allow at least **TWO** hours before to operate or switch on the system. This allows the Station to reach equilibrium with the incubator environment and reduces the risk of condensation-related problems.*

Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might affect instrument performance and void the instrument warranty.

3.2 Unpacking

The SP/MP Analyzer and SP Station are packed in a specially designed box. Retain the box and packing materials. If the system needs to be relocated or returned for repair, it should be always transported in the packaging provided. It is essential that you report any suspected damage immediately to your local ACEA representative and to the shipping agent before accepting the product.

The SP/MP Analyzer weighs approximately 7.4 kg and should be lifted with care, taking proper precautions to avoid injury.

After examining the box, place it on a flat surface in the upright position. Open the top of the box and remove the top packing material covering the SP/MP Analyzer, and lift the SP/MP Analyzer, along with the packing materials around the edges, up and out of the shipping box. Remove the packing materials from the ends of the instrument and set the instrument down carefully.

B

3.3 Space and Power Requirements

The xCELLigence RTCA SP is designed as a low-power consumption system with universal power adaptability. The total power consumption of the SP/MP Analyzer and SP Station is generally less than 10 Watts.

The SP/MP Analyzer must be placed in a location that allows easy access to the rear panel On/Off Power Switch.

Do not place anything within 10 cm of the side ventilation ports of the SP/MP Analyzer. Failure to observe this may result in overheating of the equipment and increase the risk of fire.

To facilitate easy installation and access to the SP Station, the recommended space requirement inside the incubator is 24.0 cm × 29.0 cm × 23.0 cm (W × D × H).

3.4 Environmental Requirements

The SP/MP Analyzer is designed to be used in a standard laboratory environment, and the SP Station is designed to be used in a tissue/cell culturing incubator with 98% maximum humidity without condensation or in a standard laboratory environment. Failure to follow the environmental requirements may reduce the operating life or cause damage to the instruments.

Do not place the SP/MP Analyzer on top of a cell culture incubator.

Please avoid to install the xCELLigence RTCA SP in direct vicinity of other laboratory devices which may influence the highly sensitive electronic readout of the xCELLigence RTCA SP (e.g., ultra-centrifuges, autoclaves).

3.5 Installation of the xCELLigence RTCA SP

This chapter describes the installation of the xCELLigence RTCA SP. A schematic presentation showing how to connect each component is provided in Figure 15.

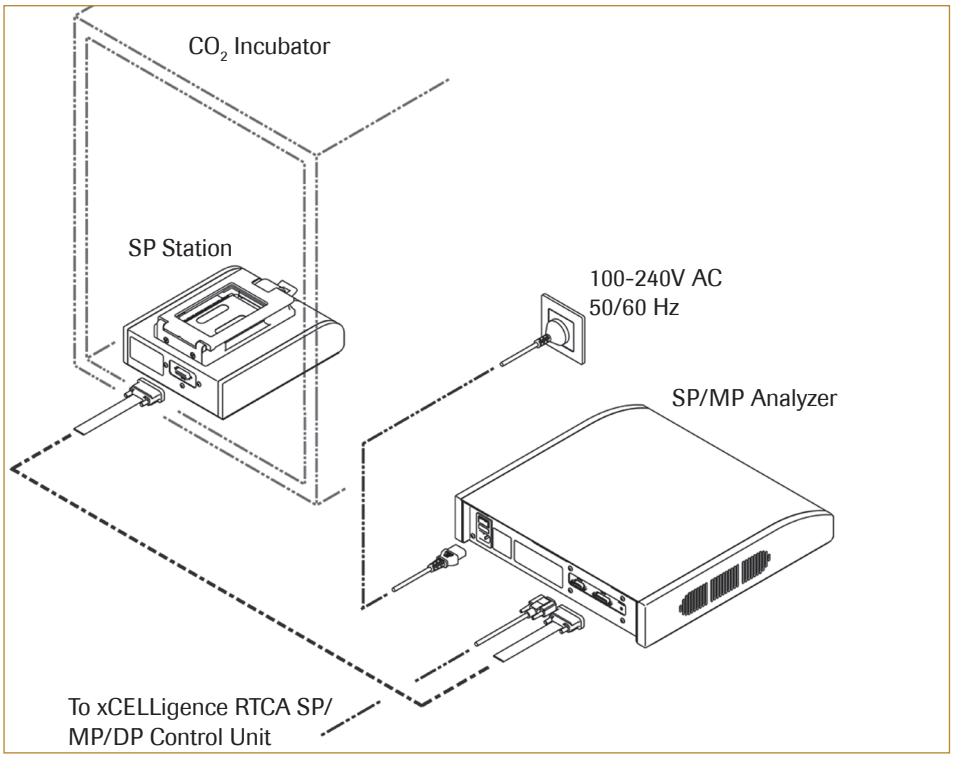


Figure 15: xCELLigence RTCA SP installation diagram.

B

	Remove instruments from boxes
1	Remove SP/MP Analyzer from the box.
2	Remove SP Station from the box.
3	Remove xCELLigence RTCA SP/MP/DP Control Unit and RTCA Software Package from the box.
	Connect the SP/MP Analyzer to the xCELLigence RTCA SP/MP/DP Control Unit
4	Locate the serial cable with two female connector ends (9-pin). Plug one end of the cable into the Serial port of the USB-Serial Converter Cable and attach the other end of the cable to the COM port on the back of the SP/MP Analyzer. Plug the Adapter into a USB port on the xCELLigence RTCA SP/MP/DP Control Unit.
	Place the SP Station into an incubator
5	Wipe the SP Station with tissue paper or a soft cloth slightly soaked with 80% alcohol or another sterilizing agent. *Avoid spraying any sterilizing agent directly on the SP Station, except Contact Pin cleaning (see Cleaning and Exchanging the RTCA Contact Pins 96)!* *Ideally, before placing the SP Station inside an incubator, it is recommended to set up the xCELLigence RTCA SP outside the incubator using a procedure similar to that being described here in section 3.5, and to perform a Resistor Plate Verification as described in section 3.6.*
6	Plug the 15-pin flat ribbon cable into the back of the SP Station, tighten the two screws on the cable, then place the SP Station in the incubator and run the flat ribbon cable from the SP Station to the outside of the incubator, making sure that the incubator door still closes.
	Connect the SP Station to the SP/MP Analyzer
7	Insert the female connector at the end of the ribbon cable into the EPS port on the rear panel of the SP/MP Analyzer. *To avoid condensation it is important to wait for at least TWO hours (after placing the SP Station inside the incubator) before running any experiments or turning on the power of the SP/MP Analyzer.*
	Attach the power cord to SP/MP Analyzer and xCELLigence RTCA SP/MP/DP Control Unit
8	Attach the DC power plug of the AC adaptor (with attached power cord) to the xCELLigence RTCA SP/MP/DP Control Unit and connect the power cord to an AC outlet. *Do NOT turn on the xCELLigence RTCA SP/MP/DP Control Unit.*
9	Attach the power cord to the SP/MP Analyzer and plug it into an AC outlet. *Do NOT turn on the SP/MP Analyzer.*
10	Wait for two hours to let the SP Station reach an equilibrium temperature without condensation.
11	Turn on the xCELLigence RTCA SP/MP/DP Control Unit.
12	Turn on the SP/MP Analyzer using the power switch located at the back of the Analyzer.

The LED on the front panel of the SP/MP Analyzer should be green.

The SP/MP Analyzer and SP Station are ready to run an assay.

The SP/MP Analyzer is pre-programmed and ready to communicate with the RTCA Software.

There is one button on the front panel of the SP/MP Analyzer which is used to perform a self-test of the instrument. A red LED will light up if the SP/MP Analyzer fails the self-test. In this case, please contact your local ACEA representative.

3.6 Resistor Plate Verification of the xCELLigence RTCA SP

Resistor Plate Verification is a very important step to verify the installation and the functionality of the xCELLigence RTCA SP. The verification process can be done outside of the incubator at room temperature. Ideally, a Resistor Plate Verification should be performed before and after the SP Station is put in an incubator. This ensures that the installation is correct, and that no condensation is affecting the SP Station after it is placed inside the incubator.

While a TWO hour equilibrium time is needed when the SP Station is first put into an incubator, resistor plate verification outside the incubator does not require this waiting period.

Start the RTCA Software

1. Having connected the cables and installed the system correctly, start the RTCA Software by double clicking the RTCA Software icon on the desktop. If the program is already open, close it and then reopen it.

2. Set up the *Exp Notes, Layout* (Turning on all the wells, right click on the *Layout* page, select all wells, then click apply button; Figure 16), and *Schedule* Pages (click add a step, set 10 sweeps with an interval of 30 sec; Figure 17). *Save* the experiment.

   For more information about setup, data format and storage, please refer to the RTCA Software Manual.

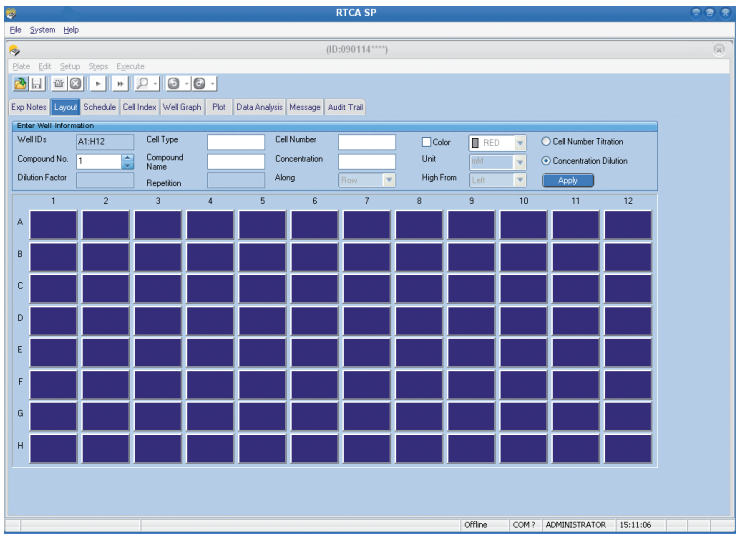


Figure 16: Setup of the *Layout* page for Resistor Plate Verification.

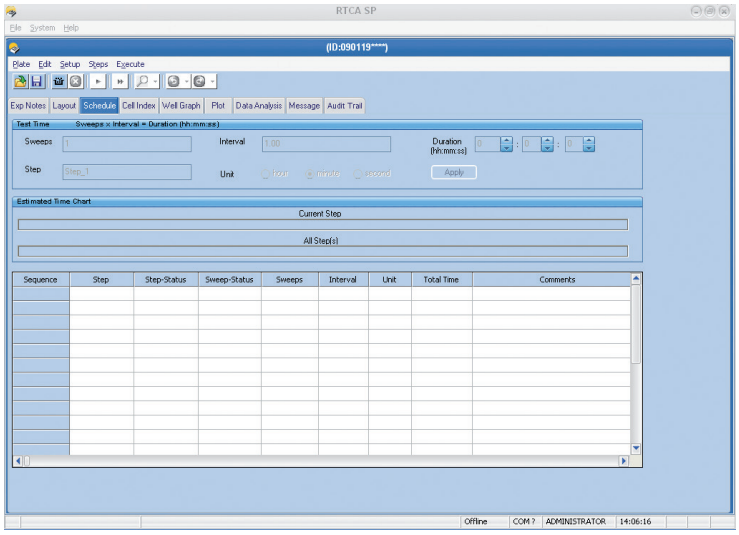


Figure 17: Setup of the *Schedule* page for Resistor Plate Verification.

B

Place the RTCA Resistor Plate 96 in the SP Station

1. Lift the clamp plate of the SP Station. Insert the RTCA Protector Shield 96 into the cradle pocket of the SP Station as shown in Figure 18.

   The RTCA Protector Shield 96 protects the RTCA Contact Pins 96, when E-Plates 96 are inserted or removed from the SP Station.

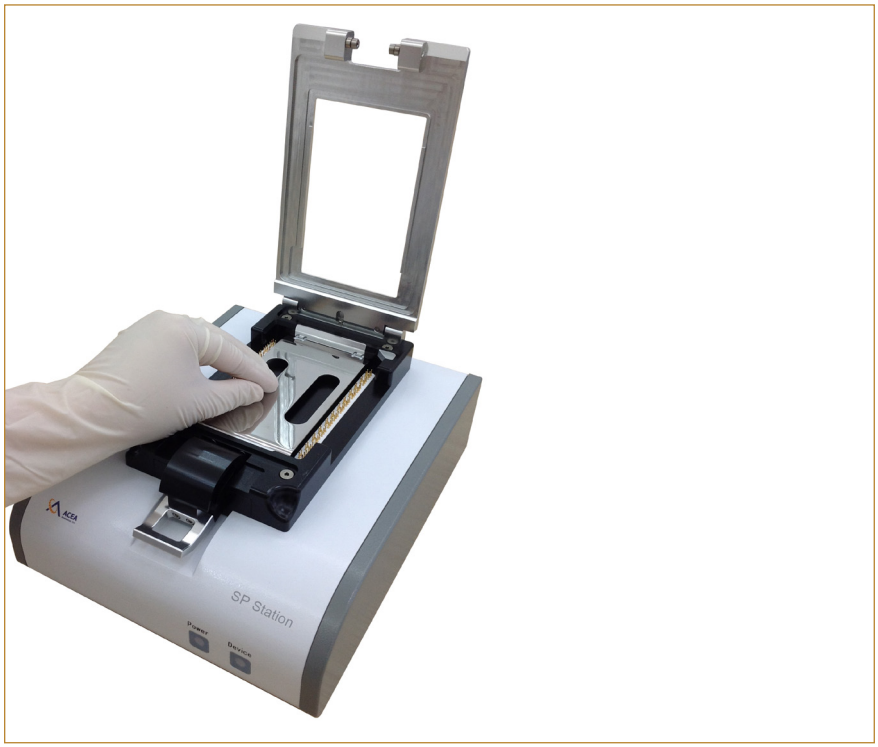


Figure 18: Insert the RTCA Protector Shield 96 in the SP Station.

2. Lift the clamp plate of the SP Station with one hand, insert one RTCA Resistor Plate 96 (with plate cover) in the cradle pocket of the SP Station (Figure 19). Ensure that the angled corners are inserted first and that the plate is fully inside the cradle pocket.

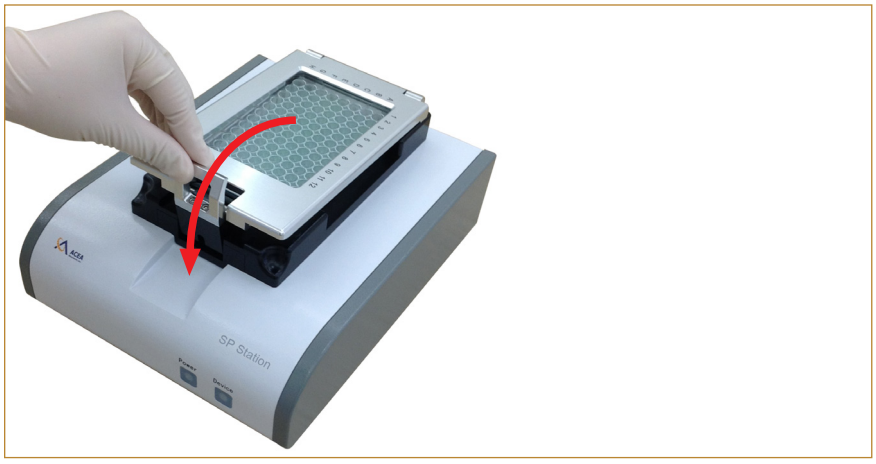


Figure 19: Insert the resistor plate and lock the clamp plate in position.

B

3 Lift the lock handle of the SP Station, so that the two bearings of the clamp plate fit into the grooves of the lock handle, then push lock handle downward to horizontal position. The clamp plate will lock the RTCA Resistor Plate 96 at the horizontal position. A green LED will light up on the front of the SP Station to indicate that a device is engaged.

Make sure that all RTCA Contact Pins 96 and the contact pads of the RTCA Resistor Plate 96 are clean and free from any dust or dirt particles. If not, use the RTCA Cleaning Kit to remove any dust.

When inserting the RTCA Resistor Plate 96 into the slot of the Station, make sure that the Resistor Plate is FULLY inside the pocket and is aligned properly with the edges of the pocket. It should be flat and without any tilt.

The Power LED (green) on the front panel of the SP Station remains on while it is connected to the switched-on SP/MP Analyzer.

Perform the test

4 Wait until the automatic scan finishes, then click *Start* to begin the test. While the test is running, notice that the bottom of the program window reads “Test Col 1,2,3, ….12”, and the status LED and communication LED on the SP/MP Analyzer will flash during the measurement.

Check the data

5 Upon completion of the test, check the *Cell Index* Page. All of the Cell Index values should be less than 0.063. Go to the *Plot* page and click *Add All*. All CI values will be displayed for the 96 wells (Figure 20).

6 In the lower portion of the *Cell Index* page, you can also view the raw scan data. The data has 8 rows by 12 columns as shown in Figure 20. The raw scan data should exhibit a repeated value pattern of 40.0 ± 2.0 (rows A and H), 67.5 ± 2.5 (rows B and G), 93.6 ± 2.9 (rows C and F) and 117.6 ± 3.2 (rows D and E).

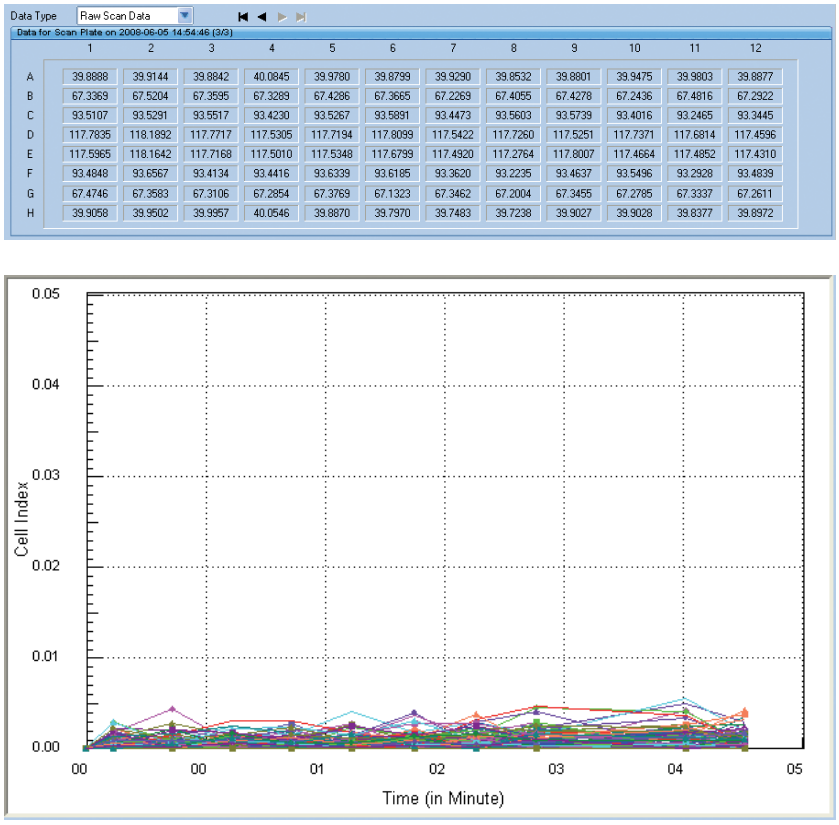


Figure 20: An example of resistor plate verification calibration raw data and Cell Index curves.

7 If the acceptance criteria described in 5. and 6. are fulfilled, the resistor plate verification is passed successfully. If the criteria are not fulfilled, please contact your local ACEA representative.

Remove the RTCA Resistor Plate 96

8 To remove the RTCA Resistor Plate 96 from the SP Station, press down lightly on the clamp plate. Then lift the lock handle gently to release, slowly allowing the lock handle to rotate upwards. Now lift the clamp plate and take the RTCA Resistor Plate 96 out (Figure 21).

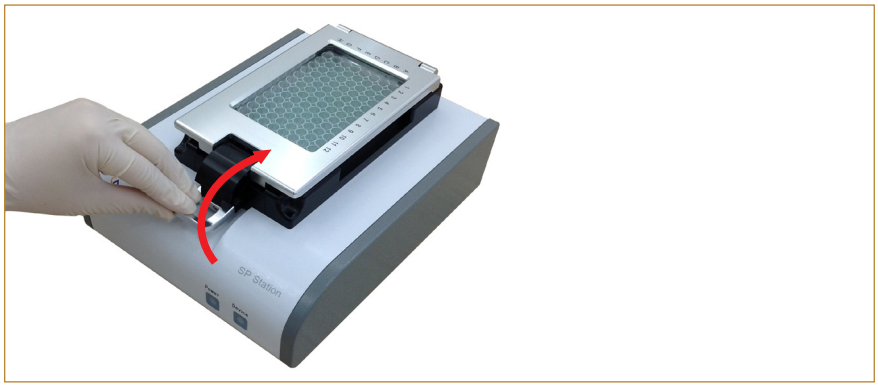


Figure 21: Removal of an RTCA Resistor Plate 96 from the SP Station.

C Operation

1. Introduction

Prior to the operation of the xCELLigence RTCA SP, please refer to the *Overview* and *System Description* sections in this manual and review the RTCA Software Manual to familiarize yourself with the system.

The operator should have some previous experience with tissue/cell culturing to handle cells and medium.

2. Instrument Start-Up and Warm-Up

The power switch is located at the back of the SP/MP Analyzer. After the system is properly installed, the power of the SP/MP Analyzer can be turned-on. The power for the SP Station is provided by the analyzer, so there is no power switch at the station side.

After placing the SP Station in the incubator, allow the system to warm up for two hours, so the SP Station reaches the same temperature as the incubator. This ensures a condensation-free station and smooth data recording, since the electrical properties of the medium and the electronics are temperature sensitive.

3. Preparing and Starting a Run on the xCELLigence RTCA SP

3.1 Running a Quick Experiment

When the SP/MP Analyzer is turned on, a self-test is performed and all LED indicators should be green.

If a RED LED lights up, it means that the instrument has failed the self-test, and you should contact a local ACEA representative immediately.

A cell titration experiment being run on The xCELLigence RTCA SP is used for illustration purpose. The procedure for this experiment includes:

- Adding culture media to the E-Plate 96
- Inserting the E-Plate 96
- Starting the RTCA Software
- Setting up *Exp Notes*, *Layout*, and *Schedule* pages
- Starting the experiment
- Removing the E-Plate 96 and adding cells to the E-Plate 96 wells
- Reinserting the E-Plate 96
- Checking and plotting the Cell Index data on the *Plot* page

1 Add Culture Media to the E-Plate 96

Add 100 µl of cell culture media to each of the 96 wells in the E-Plate 96.

For optimal results, leave the E-Plate 96 in the tissue culture hood for 30 minutes at room temperature. This ensures that the culture media and E-Plate surface achieve equilibrium.

Please consider the maximal volume of each well for your experimental setup. It is not recommended to use > 200 µl total volume for each well.

Do NOT touch the E-Plate 96 on the electrical contacts and do NOT wear powdered safety gloves. Always bear in mind that the system is especially dependent on a clean, dust-free environment.

Please avoid scratching the gold electrodes on the bottom of the wells with pipette tips. This could influence functionality of the gold electrodes.

The 96 wells within E-Plates 96 are organized in such a way that each column of 8 wells is divided into two 4-well groups. To achieve consistent and accurate data during the experiment it is necessary to use the wells within a group in the same experiment. Examples of such 4-well groups are: A1, B1, C1 and D1 as a group; E3, F3, G3 and H3 as a group, A7, B7, C7, D7 as a group, etc.

Please always use the whole plate at one time. Using only part of the wells on a plate is not recommended.

This is because, during the experiment in the incubator, the properties of the micro sensor arrays in the unused wells may be affected, so that their qualitative performance with cells can no longer be. If using only a fraction of the E-Plate 96 wells can not be avoided, it is best to fill the remaining wells with buffer (*e.g.*, PBS).

2 Insert the E-Plate 96

1. Insert the E-Plate 96 front end (with corner cut) into the cradle pocket of the SP Station. (Fig. 22)

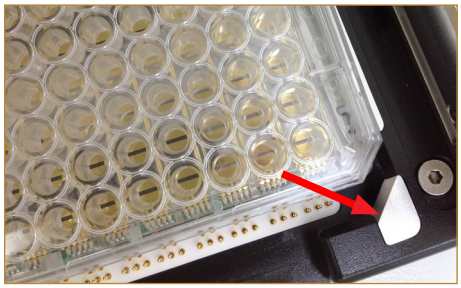


Figure 22: Correct orientation of the E-Plate 96.

2. Make sure that the plate is flat in position without any tilting. Lift the lock handle of the SP Station, so that the two bearings of the clamp plate fit into the grooves of the lock handle, then push lock handle downward to the horizontal position. The clamp plate will lock the E-Plate 96 in the horizontal position. The green light turns on when the device is correctly positioned.
3. Close the door of the incubator.

Make sure that all the 120 connector pads of the E-Plate 96 are clean and free of any dust or dirt particles. If not, wipe the connector pads with lab wipes or a cloth slightly soaked with 80% alcohol and/or dust off the connector pins with compressed air.

Make sure that all the 120 RTCA Contact Pins are clean and straight without any bending. If any damage to the Contact Pins is found, please change the Contact Pins as described in the Maintenance and Care part of this manual.

3 Start the RTCA Software

Start the RTCA Software by double clicking the RTCA Software icon on the desktop. If the program is already open, close it, and then reopen it.

4 Set up the *Exp Notes* page

Set up the *Exp Notes* page. This page records experiment-related information such as user, experiment date, experiment name, experimental purpose, experimental procedure, experimental conclusion, and the devices used in the experiment. Enter the information in the appropriate spaces. When the experiment starts, this information is automatically saved. You can also click *Browse…* to select a File Directory in which to save the experiment files.

Please refer to the RTCA Software Manual for more information about setting up the Exp Notes page.

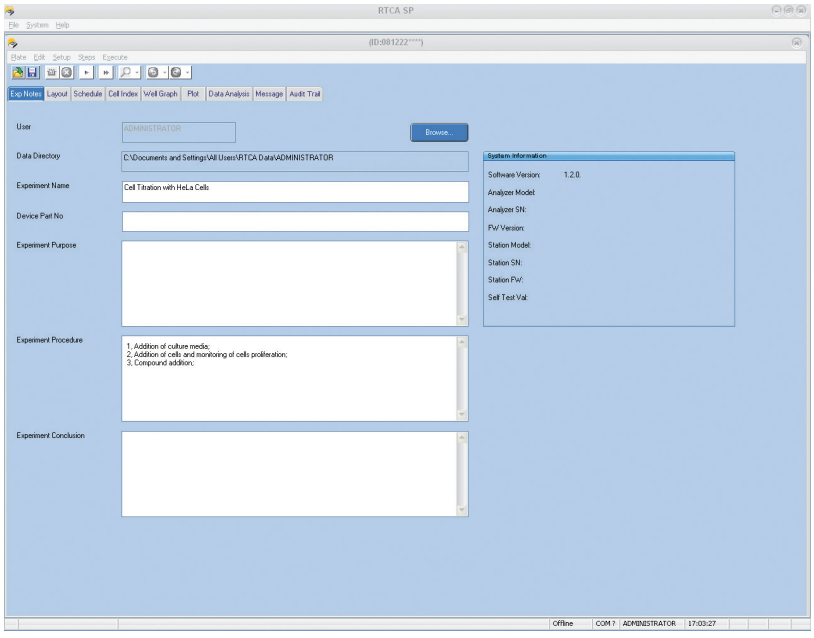


Figure 23: Example of a completed *Exp Notes* page.

5 **Set up the *Layout* page**

This page determines the experiment layout and records experiment details about each well, including: name and number of cells, name and concentration of compounds added into wells, etc.

Select the wells to be edited:

- To select a single well, move mouse cursor over the well to be selected, and left-click. The selected well is highlighted. The well ID of the selected well is automatically filled into the *Well IDs* section. If, for example, the well D1 is selected, then the *Well IDs* section reads: "D1:D1".
- To select multiple wells, move the cursor over the first well to be selected, left-click and hold the mouse. Drag the mouse across the other wells, to the last well to be selected. Release the mouse button. All selected wells are highlighted, the well's ID, for example A1:D2, is automatically filled into the *Well IDs* section, if all wells between A1 and D2 are selected.

  Note that the text in the Well IDs section has the format First well ID: Last well ID.

- To select an entire row(s) or column(s), move the mouse to the row ID (*e.g.* A or E) or column ID (*e.g.* 1 or 3), and left-click. To select adjacent multiple-rows or multiple columns, left-click and drag across row IDs or column IDs.

  Enter information into appropriate information edit boxes, and click *Apply*. For example: *Cell Type* – HeLa; *Cell Number* – 40000.

  For a detailed description of other fields, including *Compound No.*, *Compound Name*, *Concentration*, *Unit*, *Dilution Factor*, *Repetition*, *High From*, *Along* please refer to the RTCA Software Manual.

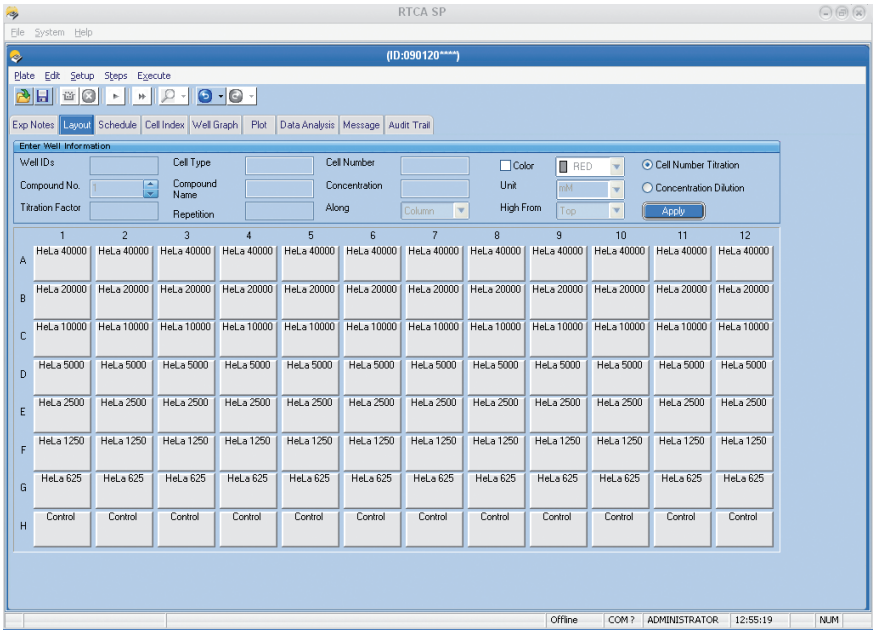


Figure 24: Example of a programmed *Layout* page.

6 **Set up the *Schedule* page**

This page is used to program the experimental procedure. Generally, experiments can be divided into multiple steps, and each step consists of one or multiple sweeps.

Each sweep is one measurement across all the selected wells. There is a pre-determined time interval between neighboring sweeps within a step. After starting a current sweep, the xCELLigence RTCA SP automatically starts to count down the time interval and starts the next sweep.

After completing all of the sweeps within a step, the xCELLigence RTCA SP does not move on to the next step until it is manually instructed to do so.

A manual intervention is always necessary to move from one step to the next one.

Generally, there should be a separate step each time an E-Plate 96 is removed. Users can manually stop a current step at any time before the system completes all pre-set sweeps and proceed to the next step. However, a current step cannot be manually stopped in the middle of a measurement sweep. Doing so may cause system instability and data loss.

- *Step 1* is typically used for background tests, where no cells are present in the wells.

  It is set as One sweep, One minute by default. The Step Status is idle, and all other buttons are blank.

  The sweep number and sweep interval for the Step 1 is pre-set to be one sweep with an interval of one minute by the software. This step is used for background measurement and should not be changed.

- *Step 2* is typically the first step after cell addition.

  To add Step 2, move the mouse to the *Steps* column on the left side of the page. Left click the *Add a Step* Icon , or go to the pull-down *Steps* menu and select *Add a Step*. Step 2 is added. Edit the *Interval* and *Sweeps* section by entering the appropriate numbers. Select the Interval *Units* in hours or in minutes. Click *Apply*, and the appropriate time intervals, with corresponding unit and sweeps are added. The *Sweep Status* is automatically set to 0. *Step Status* displays *Idle* before the start of the step and as *Test* while the experiment is in progress. When the step is completed, *Done* will be displayed. The *Sweep Status* section is updated and displays the number of sweeps that have been completed.

  Depending on the specific experiment, appropriate sweep numbers and time intervals can be set (for example, 48 sweeps, and 30-minutes intervals result in a period of 24 hours).

  It is recommended to program some sweeps in excess rather than to give a precise number, because a step can be stopped before all sweeps are completed.

6 ▶ *Step 3* typically comes after an action (for example, compound addition) on the cells.

Depending on the specific experiment, appropriate sweep numbers and time intervals can be set as described for *Step 2*. For example, to measure rapid kinetics of cell responses to compound addition, a time interval of one minute can be set. Within a step, substeps with different time intervals can be used. For example, step three can consist of 200 sweeps having one minute time intervals and another 200 sweeps having 15 minute time intervals.

▶ To remove a step, highlight the step and click the *Delete a Step* icon , or go to the *Steps* menu and select *Delete a Step*.

Please refer to the RTCA Software Manual for more information regarding the Schedule page and generation of substeps.

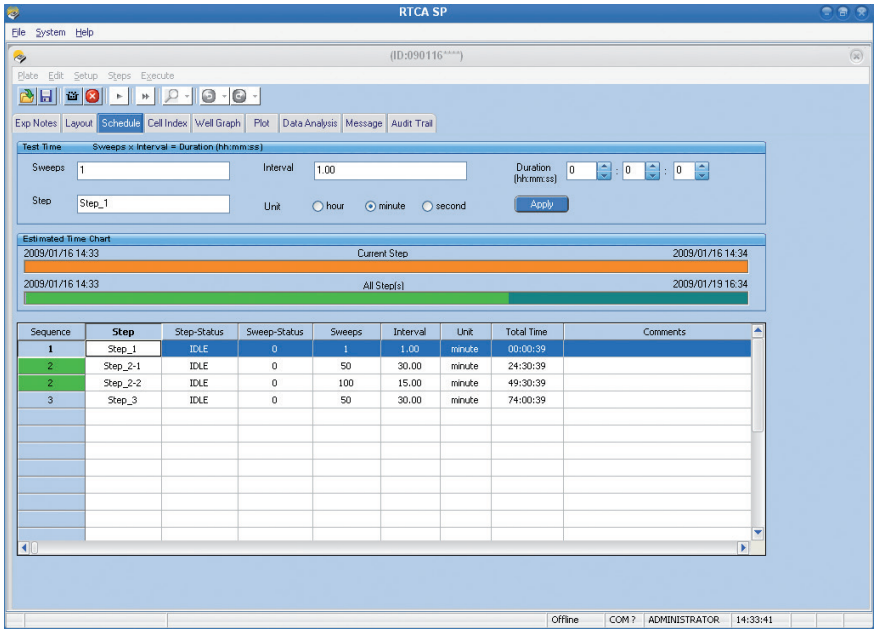


Figure 25: Example of a programmed *Schedule* page that allocates the Test Time.

7 ***Scan Plate* function**

Scan Plate is automatically performed each time an E-Plate 96 is inserted into the SP Station. In addition, Scan Plate can be performed manually.

Always use the *Scan Plate* function before you start the experiment to make sure that the connection between the E-Plate 96 and the SP Station is good. Click *Scan Plate* at the upper left corner. Then the SP/MP Analyzer will do a frequency scan over the E-Plate 96 for the selected wells. Next, go to the *Message* page to check the status. If the measured resistance value is within the expected range, a message *Plate Scanned. Connections ok.* will appear. Otherwise, you need to remove the plate and re-insert the plate into the SP Station or do some cleaning. In the meantime, a data file is also generated to record the scan plate data. By default, it is located at C:\Documents and Settings\All Users\RTCA Data\ScanPlateData\. The file has the format PlateScanning*mmddyy*.txt.

8 **Start the experiment**

Click *Start* to begin the experiment. This step measures the background impedance of cell culture media. The data is then used as reference impedance for calculating Cell Index values. During the experiment, the bottom of the program window displays *Test Col 1, 2, 3 … 12*, and the SP/MP Analyzer communication and status LED will blink during the measurement and communication with the xCELLigence RTCA SP/MP/DP Control Unit. After completion of the measurement the bottom-left of the main program window displays *Ready for Next Step. Please Click Next Step to start.*

▶▶▶

9 **Remove the E-Plate 96 and add cells to the E-Plate 96 wells**

Remove the E-Plate 96 from the SP Station by gently releasing the lock handle upward. The green light turns off.

Add 100 µl of cell suspension to each well. The cell suspension should have been properly prepared for the desired cell concentrations for the cell titration experiment.

Keep shaking for evenly distributed cell suspension so that each well has proper cell numbers.

*Leave the E-Plate 96 in the tissue culture hood for **30 minutes** at room temperature, so that the cells settle at the bottom of the well.*

10 **Reinsert the E-Plate 96 and start step 2**

Insert the E-Plate 96 as described above. Lock the E-Plate 96 to the cradle pocket by turning the lock handle to the horizontal position. The green light turns on when it is connected properly. Close the incubator door.

A Scan Plate is automatically performed. Go to the Message page to check the status.

Start step 2 by clicking the *Start Step* button. During the experiment, note that the bottom of the program window states *Test Col 1, 2,..., 12*. The bottom-left of the program window displays a countdown to the next sweep start time (for example, "0:10:30 left for 3/100[2]").

11 **Check and plot the Cell Index data**

During the experiment and between sweeps, check the *Cell Index* or *Plot* page. In the *Cell Index* page, move to different test time points (the time is the time since the start of the experiment). Cell index values at the time point are shown in boxes. In the *Plot* page, plot the cell index for a well as a function of time, by highlighting the well, and clicking *Add*. To add another well, repeat the process. By clicking *Add All*, data for every well included in the experiment is shown.

For detailed information on how to plot and analyze experimental data please refer to the RTCA Software Manual.

Typical results of a cell titration experiment generated on the *Plot* page are shown in Figure 26.

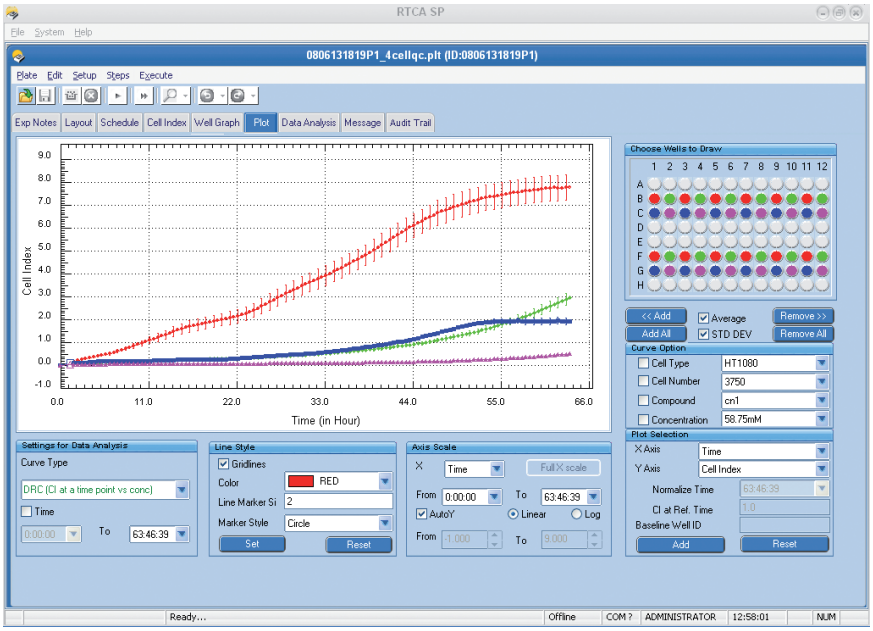


Figure 26: *Plot* page after completion of a cell titration experiment.

Charts and data on the charts can be easily exported using a right-click of the mouse.

3.2 Important Tips for Using the xCELLigence RTCA SP

The xCELLigence RTCA SP and the E-Plate 96 devices are manufactured and tested according to rigorous quality standards. Each well in E-Plates 96 is quality controlled prior to packaging and shipment ensuring that every well in every E-Plate 96 meets these quality standards.

In order to obtain optimal data and to avoid any problem with the xCELLigence RTCA SP, it is essential that you observe the following instrument handling procedures:

Always perform background measurement of E-Plate 96 with cell-free culture media.

Without cell culture media, the background measurement of E-Plates 96 cannot be performed since what is being measured is just open circuits. Similarly, the background of E-Plates 96 cannot be measured properly when the culture media already contains cells because what is being measured is not a real background.

Do not remove the E-Plate 96 from SP Station before completing the background measurement.

If the E-Plate 96 is removed from the SP Station before completion of background measurement, then those wells whose background has not been measured would be represented as an open circuit in the background data. These wells will not produce valid experimental data. The RTCA Software displays a warning message in such cases.

For the same reason, please do not remove E-Plates 96 from the SP Station when the measurement or experiment is in progress. If E-Plates 96 are removed, the impedance for these E-Plates 96 would show open-circuit data which, in turn, will display very high Cell Index figures in the *Plot* page of the RTCA Software.

*Always let the E-Plate 96 sit outside the incubator for **30 minutes** after cell addition.*

To ensure consistent data, cells need to settle down uniformly on the bottom surfaces of the E-Plate 96 wells before they are put in the incubator. Otherwise, temperature-gradient related convection in the E-Plate 96 wells would cause the cells to be distributed non-uniformly over the bottom surface of the wells, leading to substantial variation in the experiment data.

When an E-Plate 96 is taken out of the RTCA Station for compound addition or other treatment, please minimize the handling time (preferably less than 5 minutes) as cooling of the cells to room temperature will have an impact on cellular behaviour and corresponding CI values.

Add compounds gently and in as low volume as possible to avoid disturbing the cells. Use compound solution which has been pre-equilibrated to room temperature. Addition of cold compound solution may impact cellular behaviour and corresponding CI values.

Do not interrupt an experiment or a particular step while the measurement sweep is in progress.

Always wait until the current measurement sweep finishes and then stop an experiment or a particular step. Otherwise, the experiment data file may be corrupt and data cannot be retrieved.

Each step must have at least one sweep.

For the RTCA Software to run properly, at least one sweep is needed for any given step. Otherwise, the data file format may not be correct. The RTCA Software will not continue for steps without sweep numbers.

***Do not re-use** the E-Plate 96.*

The E-Plate 96 is designed for single use only. Data quality cannot be guaranteed for re-used plates since sensor surface properties of re-used E-Plates 96 would differ from the tissue-culture-compatible properties of fresh E-Plates 96. Furthermore, residual contamination in the wells might cause unreliable results.

Seed appropriate number of cells into the wells of the E-Plate 96.

Before running any assay on the xCELLigence RTCA SP, a cell titration experiment is advisable to test cell proliferation for a range of cell numbers. This approach helps to determine the appropriate number of the cells for the assay. If cell number is too high or too low, it would affect the data quality generated from the xCELLigence RTCA SP.

Thoroughly mix the cell suspension when seeding cells into the wells.

Please be aware that under some circumstances (e.g., extremely low cell numbers) experimental data might vary between wells in the center and wells on the edge of E-Plates 96 (so-called "Edge Effect"), which is as well a commonly known phenomenon of conventional microtiter plates.

Cells will settle to the bottom of the cell-suspension tube unless repeatedly mixed. In order to add a consistent number of cells to each well, it is essential to have cells uniformly (and randomly) suspended in the tube. Thus it is important to mix the cell suspension in the tube before adding cells to each well.

Make sure that all the 120 connector pads on the backside of the E-Plate 96 are clean and free of dust/dirt particles.

Clean the connector pads (located on small, green PCB boards on the bottom of the E-Plate 96) are essential for reliable contacts between E-Plate 96 and SP Station. If connector pads are dirty, wipe the pads gently with 80% alcohol-soaked tissue (paper/cloth). Do not press too hard on such pads to avoid damaging the connection between the small PCB boards to the plate.

Always check the Scan Plate data when the E-Plate 96 is re-positioned back on the SP Station after being removed. "Connections ok" will be displayed if there is no connection problem after each Scan Plate operation, including auto-scan after plate repositioning.

After the *Scan Plate operation*, please check the *Message* page for scan results to see whether some wells have connection problems. When some wells show a problem, first try one of two following steps to resolve the issue:

1. With the E-Plate 96 on the SP Station and in the locked position, you may want to press the plate down a couple of times on the plate. This action can sometimes remove the dust between the RTCA Contact Pins 96 and the connector pads, or reset the RTCA Contact Pin 96 connection to allow better contact. You can then do a *Scan Plate* which may show that the problematic well is now OK.
2. The second option is to remove the E-Plate 96 and re-position it on the SP Station. Before you re-position the E-Plate 96, please check to see whether the E-Plate connector pads are clean. Alternatively, you may just clean the connector pads of the E-Plate 96 before re-positioning it for another *Scan Plate* test.

If the problem persists you may want to continue the experiment with this E-Plate 96, or you may want to try again with a new E-Plate 96 (assuming that the cells have not been added). You should report such incidents to your local ACEA representative and provide the serial number of the E-Plate 96 and the appropriate data file (for details, see *Troubleshooting*).

Turn off the SP/MP Analyzer if the system is not used for several days or longer.

As with any electronic equipment, the xCELLigence RTCA SP should be switched off when not in use. This will prolong the operating life of the equipment and save electricity.

D Maintenance and Care

1. General Maintenance

The xCELLigence RTCA SP is maintenance-free.

There are no user serviceable parts inside the SP/MP Analyzer or the SP Station.

If the system will not be used for a long period of time (a month or more), pack the SP/MP Analyzer and the SP Station into the original plastic bags inside the shipment boxes.

When you are ready to use the instrument again, please refer to the *Installation* section for details on unpacking and installation.

*The surface of the SP/MP Analyzer and SP Station should be cleaned and wiped with a towel or tissue paper that has been **lightly** soaked in 80% alcohol. Ensure that this is done for the SP Station to be placed inside an incubator.*

Avoid spraying any sterilizing agent directly on any part of the xCELLigence RTCA SP, except Contact Pin cleaning (see Cleaning and Exchanging the RTCA Contact Pins 96)!

2. Cleaning Instructions

2.1 General Cleaning

General cleaning of the xCELLigence RTCA SP is simple. Use some soft cloth or fiber free tissue paper moistened slightly with 80% alcohol, gently wipe off any dust or contaminants from the surface of the SP Station. A small air blower can also be used to help remove any dust.

Be careful not to touch the RTCA Contact Pins 96. They must be cleaned with a special brush.

Do not soak the cloth or tissue paper excessively. In particular, do not let any liquid drip on to the RTCA Contact Pin contacts. Clean up any liquid spilled on the RTCA Contact Pin cradle pocket surface immediately. Any liquid spilled on the RTCA Contact Pin contacts may affect the reliability of the electrical connection.

Decontamination of the RTCA Station, SP/MP Analyzer and xCELLigence RTCA SP/MP/DP Control Unit

For decontamination of the RTCA Station, SP/MP Analyzer and xCELLigence RTCA SP/MP/DP Control Unit the user has to follow the governmental guidelines for the inactivation of the organisms used in the experimental environment.

The following compositions have been tested for being compatible with the surfaces of the RTCA Station, the SP/MP Analyzer and the xCELLigence RTCA SP/MP/DP Control Unit and cables:

- 80% Ethanol
- A mixture containing propan-1-ol 450 mg/g, propan-2-ol 250 mg/g, ethanol 47 mg/g

This mixture is available for instance from Bode Chemie under the tradename Bacillol® AF.

This mixture is fungicidal, tuberculocidal, mycobactericidal, virucidal against enveloped viruses (including HBV, HIV, HCV), Adeno virus, FCV, Papova virus and Rota virus when used as described in the supplier's instructions.

Other sterilizing agents, such as oxidizing agents or strongly alkaline or acidic substances used for decontamination may damage the surfaces of the RTCA Station, the SP/MP Analyzer and the xCELLigence RTCA SP/MP/DP Control Unit or may interfere with their function.

2.2 Further Recommendations

The xCELLigence RTCA SP is a precision instrument that requires care to enable it to perform consistently at optimum level. General decontamination is highly recommended by wiping out any dust or mold growing on SP Station surfaces.

This cleaning and care also applies to the tissue culture incubator that holds the SP Station. It is important to prevent any mold from growing inside the incubator, this will probably affect the SP Station. It is suggested to use copper alloy based incubators and do regular cleaning to prevent any mold growth inside the incubator.

3. Cleaning and Exchanging the RTCA Contact Pins 96

The RTCA Contact Pin 96 contacts provide an important signal path from the microsensor array in the E-Plate 96 to the control and measurement circuits. To guarantee the proper function of the SP Station, clean the RTCA Contact Pins regularly. In addition, whenever a particular RTCA Contact Pin shows significantly higher contact resistance compared to the others, that pin should be cleaned. If one of the CI values during RTCA Resistor Plate 96 verification tests is higher than 0.063, the RTCA Contact Pins 96 should either be cleaned or replaced.

The RTCA Contact Pin 96 cleaning procedure takes 10 to 15 minutes and recommended to ensure helpful obtaining consistent optimum experimental data. Regular cleaning every three months, or whenever contaminants are present on the RTCA Contact Pin contacts, produces the most reproducible and accurate measurements.

1. Remove the RTCA Protector Shield 96 from the cradle pocket. Blow the entire surface of the SP Station using the small dust blower which comes with the xCELLigence RTCA SP. If compressed air and nozzle are available, it can also be used. Use a pressure less than less than 15 psi and blow air gently.

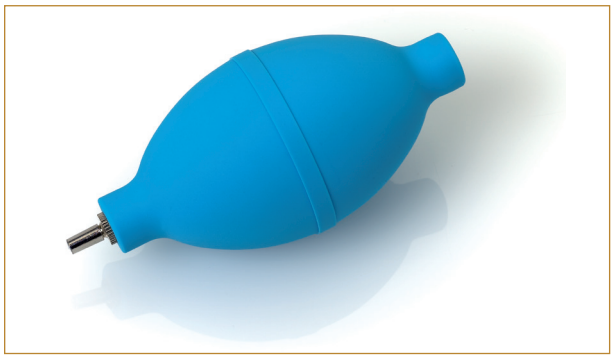


Figure 27: Dust Blower.

2. Brush the RTCA Contact Pin field with the nylon brush. There are two rows of the Contact Pins and each row contains 12 groups of Contact Pins. The procedure for cleaning one row of Contact Pins is as follows:

   (a) Position the nylon brush to cover 3 groups of Contact Pins.

   (b) Brush across the tips of the Contact Pins gently in only one direction at a time, as shown (up to down) in Figure 28.

   (c) Repeat the motion in (b) at least ten times.

   (d) Position the brush to cover and clean the next 3 groups of Contact Pins.

   (e) Repeat above process (a) through (d) to cover the entire row of Contact Pins.

   (f) Use the air blower to remove any dust from the surface close to this row of Contact Pins.

   (g) Repeat this routine (a) through (f) in the opposite direction (down to up).

   When using blower to remove dust, always remember to blow the air towards the outside of the cradle area.

▶▶▶

2. Repeat the above procedure to clean the other row of Contact Pins.

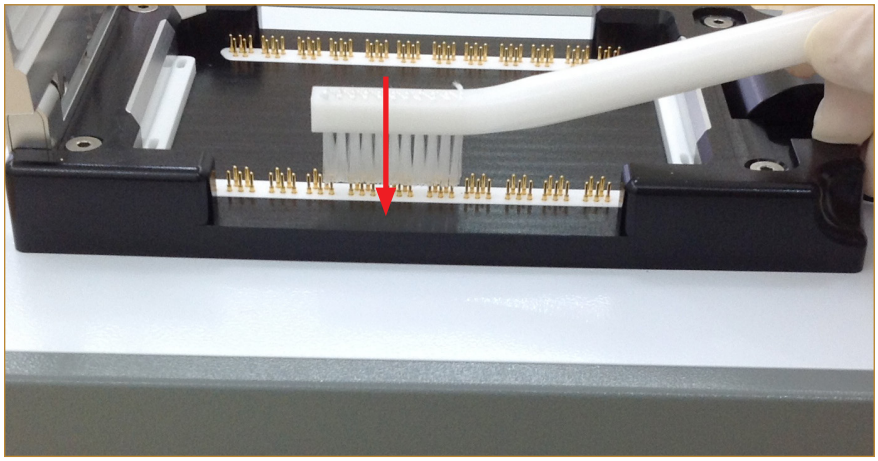


Figure 28: Using the Nylon Brush to clean the RTCA Contact Pins 96 in only one direction at a time (up to down in the Figure).

Before using the nylon brush to clean the Contact Pins, please make sure the brush is clean. If you are not sure, please use the blower to blow the brush from each side for at least 5 times.

3. Use the air blower to blow the surface around the RTCA Contact Pins 96 to remove any dust.
4. Before reinserting the RTCA Protector Shield 96, be sure that all RTCA Contact Pins have not come loose. They should be straight and at the same height.
5. Insert the RTCA Protector Shield 96.

Decontamination of RTCA Contact Pins 96

1. Remove RTCA Protector Shield 96 from the SP Station. Clean the RTCA Contact Pins 96 with the brush and use the rubber dust blower (from RTCA Cleaning Kit) to remove any dust particles from the Contact Pins, following the protocol described in this section.
2. Spray 80% ethanol with a spraying bottle on all Contact Pins, two times on each location. Make sure that all the Contact Pins are covered with ethanol. The total volume of ethanol for spraying the two columns of Contact Pins should not exceed 3 ml.
3. Wait at least 30 minutes at room temperature for the ethanol to evaporate and let the RTCA Contact Pins 96 dry completely.

   Since the brush is used for cleaning the dust particles from the Contact Pins, the brush should also be decontaminated with ethanol. Fill a container with 80% ethanol. Then soak the brush in the container for 5 minutes. Take the brush out, shake the excess ethanol from the bristles and wait for the brush to dry.

Exchange the RTCA Contact Pins 96

In case of a damaged or failed RTCA Contact Pin, the respective pin can be exchanged with a new one using an easy and quick process.

Defective RTCA Contact Pins 96 should only be replaced according to the instructions described in this manual.

Spare RTCA Contact Pins 96 are included with xCELLigence RTCA SP. If more RTCA Contact Pins 96 are needed, please contact your local ACEA representative.

The wells concerned (*e.g.*, G10) can be correlated with the RTCA Contact Pin that is likely to be responsible for contact problems with the aid of Figure 29:

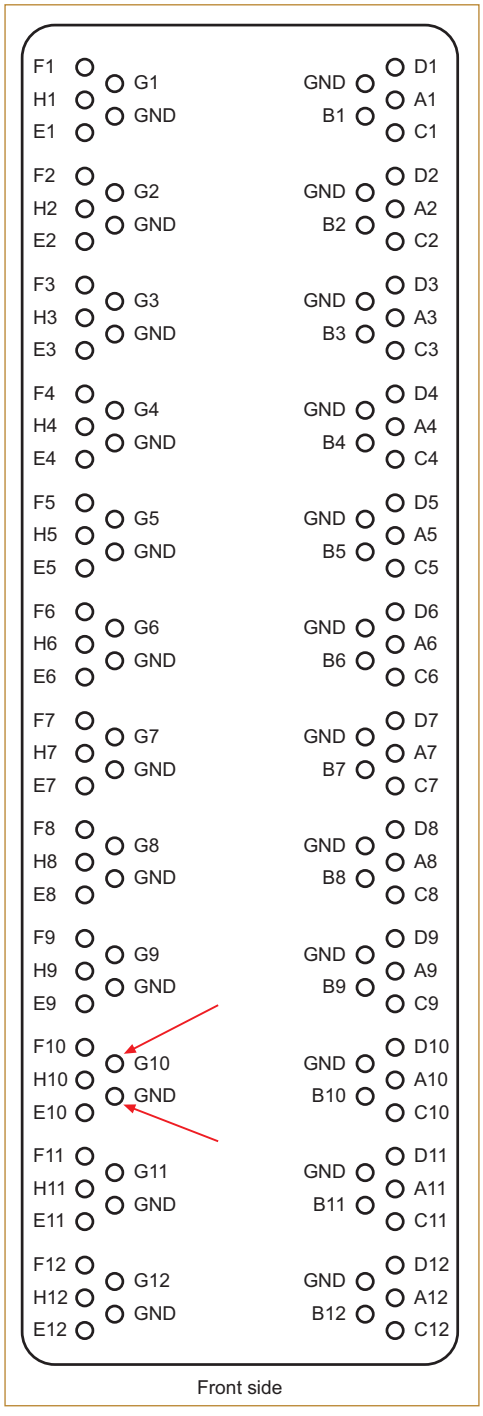


Figure 29: RTCA Contact Pin fields.
The front side of the SP Station is where the lock handle is located.

For example, if well G10 would have a contact problem, the RTCA Contact Pin for G10 and/or the corresponding Pin for ground (GND) would have to be exchanged. The positions are marked by arrows in Figure 29.

If well G10 has a contact problem but wells E10, F10 and H10 do not, then replace the RTCA Contact Pin for G10.

If well G10 has a contact problem and wells E10, F10 and H10 do also, then replace the GND Pin for the group E10 to H10.

The following procedure is suggested for exchanging the RTCA Contact Pins 96:

1. Remove RTCA Protector Shield 96 from the cradle pocket of the SP Station. Blow any dust away from the entire surface of the SP Station.

2. Use the pliers (Figure 30) to clamp and fasten the RTCA Contact Pin gently. Then vertically extract the defective RTCA Contact Pin as shown in Figure 31. Be careful not to press other adjacent contact pins.



Figure 30: RTCA Contact Pin Replacement Tool

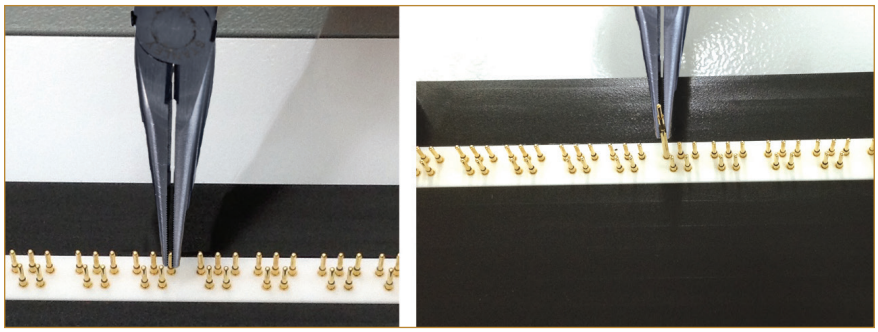


Figure 31: Extraction of an RTCA Contact Pin

3. Carefully insert a new RTCA Contact Pin 96 vertically into the receptacle hole, without bending, then use the RTCA Contact Pin Insertion Tool to push down the RTCA Contact Pin 96 vertically down close to the bottom surface. The RTCA Contact Pin 96 should be at the same height as the other RTCA Contact Pins 96 (Figure 32).



Figure 32: Replacing RTCA Contact Pins 96

4. Use the dust blower to blow the surface around the RTCA Contact Pins 96 to remove any dust.

5. Insert the RTCA Protector Shield 96.

4. Exchanging Fuses

In case there is a need for fuse replacement such as a power surge, observe the following steps to replace the fuse. If the new fuse gets burned again, please unplug the SP/MP Analyzer power and contact your local ACEA representative.

The SP/MP Analyzer is equipped with 2-pole fusing. Prior to servicing fuses, ensure that the power switch is in the off "O" position and disconnect the incoming AC power cord. Replace fuses only with the type and rating shown on the Power/Fuse label on the rear panel of the SP/MP Analyzer.

⚠ *Take care to use only Type S500 5 × 20 fast acting fuses with a 250V, 2AL (low breaking capacity) rating. Failure to do so may result in the potential risk of fire and/or personal injury.*

1. Using a small, flat screwdriver, depress the snaps on both sides of the fuse drawer.



Figure 33: Unlocking the fuse drawer.

2. Pull the fuse drawer out of the AC Receptacle. The fuses should remain connected to the drawer.



Figure 34: Pulling out the fuse drawer.

3. Remove the old fuses from the drawer.

4. Insert new fuses as shown.



Figure 35: Inserting new fuses in the drawer.

5. Press the fuse drawer, along with the fuses, into the AC Receptacle. Ensure that both ends of the drawer snap completely into place.



Figure 36: Inserting the fuse drawer.

D

E Appendix

1. Troubleshooting

1.1 I started an experiment and there was an error message, “Cannot find instrument”, what happened?

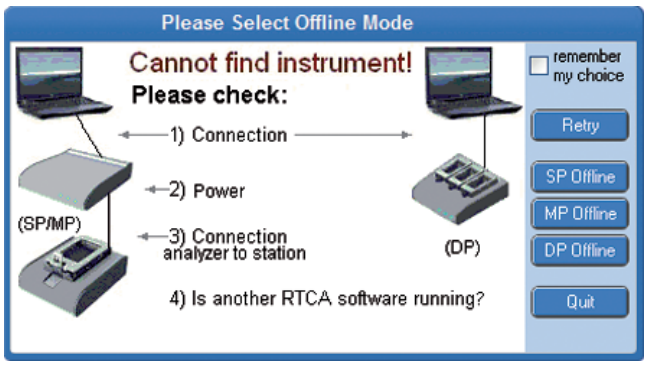


Figure 37: “Cannot find instrument” error message.

The xCELLigence RTCA SP/MP/DP Control Unit failed to communicate with the SP/MP Analyzer. This message occurs right after one clicks the *RTCA Software* icon.

Please check SP/MP Analyzer power and if the RS232 serial cable, linking the xCELLigence RTCA SP/MP/DP Control Unit to the SP/MP Analyzer (COM port), and the cable connection between SP/MP Analyzer and SP Station, are securely connected. Try the following steps:

1	Turn off the SP/MP Analyzer.
2	Disconnect the serial cable from the SP/MP Analyzer and from the xCELLigence RTCA SP/MP/DP Control Unit.
3	Reconnect the cable.
4	Disconnect the flat cable from the SP/MP Analyzer and from the SP Station.
5	Reconnect the flat cable.
6	Turn on the SP/MP Analyzer.
7	Click *Retry*.

1.2 I started an experiment and there was a sound and error message, “No Station”, what happened?

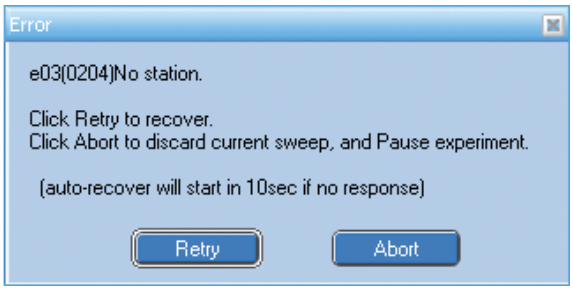


Figure 38: “No station” error message.

The SP/MP Analyzer failed to communicate with the SP Station. Please check the SP/MP Analyzer EPS cable, linking the SP/MP Analyzer to the SP Station, for secure connection. Do the following:

1. Turn off the SP/MP Analyzer.
2. Disconnect the EPS cable from the SP/MP Analyzer and reconnect the cable.
3. Disconnect the EPS cable from the SP Station and reconnect the cable.
4. Turn on the SP/MP Analyzer.
5. Click *Retry*.

1.3 I started an experiment. There was an error message, “ACK communication lost.” What happened?

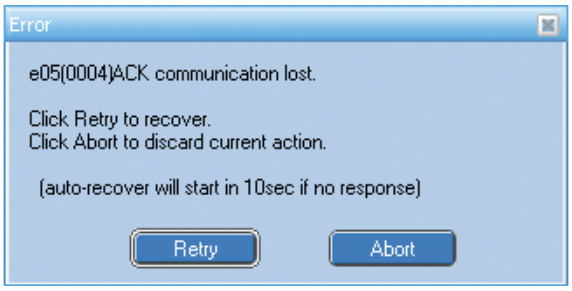


Figure 39: “ACK communication lost” error message.

The xCELLigence RTCA SP/MP/DP Control Unit failed to communicate with the SP/MP Analyzer. Please make sure that SP/MP Analyzer power is on and the RS232 serial cable, linking the xCELLigence RTCA SP/MP/DP Control Unit to the SP/MP Analyzer (COM port), is securely connected. Try the following steps:

1. Turn off the SP/MP Analyzer.
2. Disconnect the cable from the SP/MP Analyzer and from the xCELLigence RTCA SP/MP/DP Control Unit.
3. Reconnect the cable.
4. Turn on the SP/MP Analyzer.
5. Click *Retry*.

If this error message appeared in the middle of an experiment and you clicked the *Abort* button, try the following steps:

1. Turn off the SP/MP Analyzer.
2. Disconnect the cable from the SP/MP Analyzer and from the xCELLigence RTCA SP/MP/DP Control Unit.
3. Reconnect the cable.
4. Turn on the SP/MP Analyzer.
5. Click *Continue* button in the top left corner of the RTCA Software.

1.4 How do I reinitialize the xCELLigence RTCA SP after a power failure?

1. When the power is restored, restart the xCELLigence RTCA SP/MP/DP Control Unit. Do not open the RTCA Software at this time.
2. Turn off the SP/MP Analyzer. Wait for 2-3 seconds, and then turn it back on.
3. Open the RTCA Software and locate the data file that was generated before and during the power failure. Open the file.
4. Click the *Continue* button in the top left corner of the RTCA Software. The experiment starts where it left off when power was interrupted.

1.5 The Cell Index signal is significantly lower than usual. Why?

You should take the E-Plate 96 out of the incubator and check the cell status (cell number, cell distribution and morphology) under a microscope. The following are possible reasons:

- Maybe the initial seeding cell number was not correct.
- Maybe you forgot the step of "leave the E-Plate 96 at room temperature for about 30 minutes" after the cells were seeded. If that step was not done, then cell distribution might not be uniform over the well surface. The cell index signals would be low, especially for the corner wells.
- Maybe there is some type of contamination and cells are dying.
- Maybe the cells were harvested from an over-confluent flask. Our experience is that if the cells in the flask reached a confluence over 80%, then the cells may not be in the Log phase and the cell status may not be ideal. Such cells may give a low cell index signal when seeded into an E-Plate 96.
- Maybe there is something wrong with the incubator. One likely symptom of incubator problems is that the cell index is lower on edge wells than on the center wells, or the cell index drops to nearly zero faster on the edges than the center wells. The problems with incubators may include CO_2 level, temperature control, humidity control and certain chemicals released from the incubator. We have encountered several instances where the incubator itself was presenting to be "toxic" or affecting cell growth. Sometimes a simple but thorough cleaning of the incubator interior may be sufficient to solve the problems. Alternatively, simply running the experiment in another incubator may solve the problems.

Please make sure, when removing the E-Plate 96 from the incubator, that the instrument is not performing a measurement. If the instrument is *waiting for next sweep*, make sure that you have enough time to check the cells under a microscope. If you do not have time, you may have to stop the current step and then take the device out of the incubator for assessment. Then *Resume* the experiment after putting the E-Plate 96 back in.

1.6 The Cell Index signal decreases with time, but there was no compound added. Why?

Remove the E-Plate 96 from the incubator and check the cell status (cell number, cell distribution and morphology) under a microscope. Check for the following:

- Contamination resulting in cells dying.
- Culture media that is too old to support the cell growth, because the experiment is too long.
- Culture media quantity is insufficient to support cell growth, due to too much evaporation.
- Maybe there is something wrong with the incubator. One likely symptom of incubator problems is that the cell index is lower on edge wells than on the center wells, or the cell index drops to nearly zero faster on the edges than the center wells. The problems with incubators may include CO_2 level, temperature control, humidity control and certain chemicals released from the incubator. We have encountered several instances where the incubator itself was presenting to be "toxic" or affecting cell growth. Sometimes a simple but thorough cleaning of the incubator interior may be sufficient to solve the problems. Alternatively, simply running the experiment in another incubator may solve the problems.

Please make sure, when removing the E-Plate 96 from the incubator, that the instrument is not performing a measurement. If the instrument is *waiting for next sweep*, make sure that you have enough time to check the cells under a microscope. If you do not have time, you may have to stop the current step and then take the device out of the incubator for assessment. Then *Continue* the experiment after putting the E-Plate 96 back in.

1.7 The Cell Index signal is significantly higher than usual. Why?

Remove the E-Plate 96 from the incubator and check the cell status (cell number, cell distribution and morphology) under a microscope. It is possible that the initial seeding number was high.

Please make sure, when removing the E-Plate 96 from the incubator, that the instrument is not performing a measurement. If the instrument is *waiting for next sweep*, make sure that you have enough time to check the cells under a microscope. If you do not have time, you may have to stop the current step and then take the device out of the incubator for assessment. Then *Continue* the experiment after putting the E-Plate 96 back in.

1.8 I did a background measurement. The RTCA Software indicated that many wells may have background problems. What should I do?

Please remove the E-Plate 96 from the SP Station, ensure that the contact pads on the E-Plate 96 are clean and re-insert the E-Plate 96. Then perform *Scan Plate*. Check the *Message* page for results to see whether you have many wells showing connection problems. You can also check the plate scanning data shown on the *Cell Index* Page. You can check whether any measured well values show an open circuit (*i.e.*, the resistance values are abnormally high, for example, more than 500 ohm) or are negative. If the scan-plate *Message* page shows *Plate Scanned. Connections ok*, then you should be able to re-start the experiment. Note that the RTCA Software may still indicate that a few wells may have background problems since the software has strict criteria for determining whether a well has a good background. A well that is indicated as "may have background problems" does not always cause an experimental problem.

If the message indicates connection problems with many wells or if the plate-scanning data file shows many wells having open circuits, there may be problems with the E-Plate 96 or SP Station or both. Please perform an additional *Scan Plate* after removing the E-Plate 96 in question from the SP Station and then re-inserting/repositioning it in the Station. Check the *Scan Plate* results again, as described above, to see whether the results for the wells concerned are similar to previous results. If problems do not occur in the 2nd *Scan Plate*, experiments can be continued. The initial issue may be related to the insertion or positioning of the E-Plate 96 into the SP Station.

If the results for wells with connection problems or open-circuit wells are similar to previous results, the E-Plate 96, the SP Station or both have problems.

First, please check to see whether the E-Plate 96 or SP Station is damaged. If so, contact your local ACEA representative.

Second, if there is no visible damage, see whether all connector pads on the E-Plate 96 are clean and free of dust/dirt particles. If not, clean the connector pads on the E-Plate 96 with a tissue wetted with 80% alcohol. After this cleaning, re-insert and reposition the E-Plate 96 for another *Scan Plate* test. If the number of open-circuit wells is the same as before, then please report the problems to your local ACEA representative.

1.9 I placed an E-Plate 96 into the cradle of the SP Station. The Device Indicating LED did not turn green. What should I do?

First, check whether an RTCA Protector Shield 96 is placed properly inside the cradle pocket.

Second, check whether the E-Plate 96 has a 96 × lid covering the E-Plate 96.

Third, after checking the above two items, remove the E-Plate from the SP Station. Then turn off the SP/MP Analyzer. After 5 seconds, turn on the SP/MP Analyzer. Then re-insert the E-Plate 96 into the cradle of the SP Station and close the clamp plate with the handle in front of the cradle. Check whether the Device Indicating LED turns green. If the LED did not turn green (no light), then please contact your local ACEA representative.

1.10 I am contacting the local ACEA representative or ACEA Technical Support. What data files should be sent?

In case of inquiries or complaints, please ALWAYS SEND the experiment file (*.plt), including the experiment data and information. Alternatively, please use the Problem Report function, which is available under the *File* pull-down menu in RTCA Software, to create an Problem Report. The RTCA Software will provide an option that allows you to remove any confidential information from your experiment. Do not include any confidential information in the files that are sent. Please remove any information that is confidential. After the Problem Report is created, please SEND the Problem Report file (Err_YYMMDD****.zip).

2. Ordering Information

ACEA Biosciences Service and Support

At ACEA, we are committed to providing innovative, high-quality products and excellent customer service. For a complete overview of our products or to find a local representative, please visit www.aceabio.com

xCELLigence RTCA SP/MP Analyzer (SP)		**Cat. No. 00380601510**
SP Station		**Cat. No. 05229057001**
xCELLigence RTCA SP/MP/DP Control Unit		**Cat. No. 05454417001**
RTCA Protector Shield 96		**Cat. No. 05288967001**
RTCA Resistor Plate 96		**Cat. No. 05232350001**
RTCA Frame 96		**Cat. No. 05232392001**
E-Plate 96	6 Units	**Cat. No. 05232368001**
	6 x 6 Units	**Cat. No. 05232376001**
E-Plate Insert 96 (1 x 6 units) (36 16well inserts)		**Cat. No. 06465412001**
E-Plate Insert 96 Accessories (1 x 6 Units) (6 Rcvr Plates & Lids)		**Cat. No. 06465455001**
RTCA Contact Pins 96 (20 units)		**Cat. No. 05232384001**
RTCA Software Package		**Cat. No. 05454433001**

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