REMS KIT

TEER Measurements for Robotic Systems

INSTRUCTION MANUAL

Serial No._____

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World Precision Instruments

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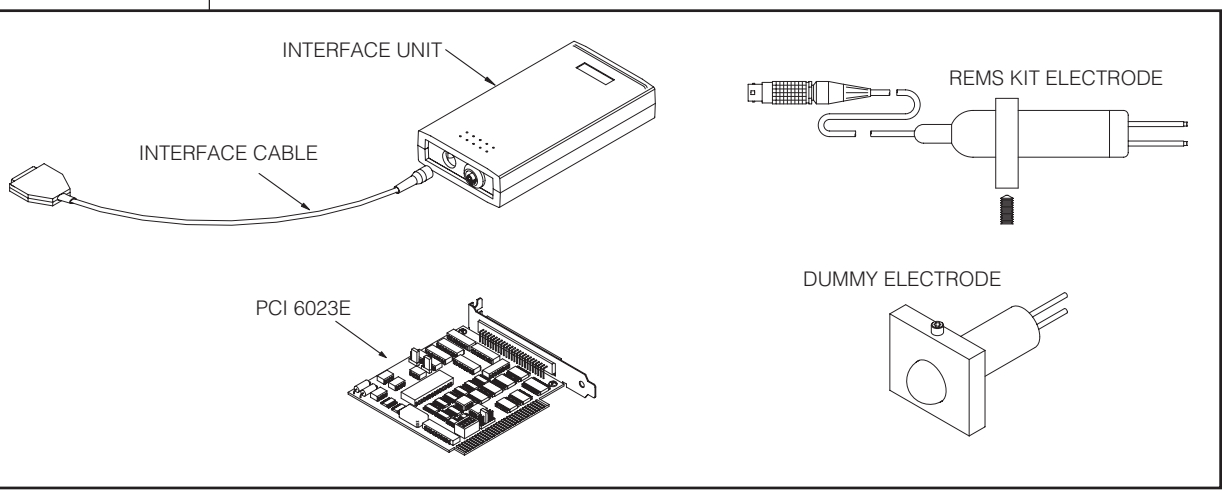
INTRODUCTION

The REMS KIT is designed to perform routine TEER (Trans-Epithelial Electrical Resistance) measurements in tissue culture research. Based on the original design principles of WPI's popular hand-held TEER measurements system (*i.e.,* **EVOM**), the REMS KIT has been developed specifically for integration with automated High Throughput-Screening (HTS) applications where TEER measurements are made robotically on cell cultures grown on 24-well and 96-well microplates. The REMS KIT is designed to be used with Windows 98 and Windows NT. Any Windows-compatible programming language that uses the DDE protocol can be used to communicate with the REMS KIT software. The source code for programs written in Visual Basic and LabVIEW, for example, is included.

REMS KIT DESCRIPTION

Parts List

1. **REMS KIT electrode.** The REMS Kit electrode is designed to take TEER measurements using the Falcon 24-well microplate, the Corning Costar 24-well microplates, and the Millipore Multiscreen™ Caco-2 96-well microplate. The electrode plugs into the REMS interface unit. The rectangular block attached to the REMS Kit electrode is designed to be mounted on a robot.
2. **Dummy electrode.** The Dummy electrode is used in place of the actual electrode for testing the positioning of the robotic arm and electrode.
3. **REMS Interface Unit.** The REMS interface unit generates and processes the electrical signals used with the electrode and the computer.
4. **PCI 6023E data acquisition board.** This board is placed inside the computer and is used to generate and process electrical signals.
5. **REMS KIT software CD.**
6. **Instruction Manual.**
7. **REMS Interface Unit Cable.** This cable connects the REMS interface unit with the data acquisition card of the computer.



Recommended Equipment

1. 266 MHz Pentium or faster computer with over 64 Mb of RAM, a CD-ROM reader and a open PCI slot.
2. Windows 98 or Windows NT operating system.
3. Windows-compatible programming language that uses the DDE communication protocol (for example, Visual Basic, C, or LabVIEW).

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. Please read the section entitled “Claims and Returns” on the Warranty page of this manual. Please call WPI Customer Service if any parts are missing.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100 mm (four inches) of shock absorbing material. Please read the section entitled “Claims and Returns” on the Warranty page of this manual.

SETUP

1. Place the NI-DAQ CD that came with the PCI-6023E data acquisition card into your computer. Select install NI-DAQ and follow the directions on your screen.
2. Turn off your computer. Install the PCI-6023E into an empty slot on your computer. Turn your computer back on. Windows should recognize the PCI-6023E and install the drivers for it.
3. Place the REMS Kit software CD into the CD-ROM reader on your computer. Double click on setup. Follow the directions on your screen.
4. Connect one end of the REMS interface Unit cable to the computer and connect the other end to the REMS interface unit.
5. Connect the REMS Kit electrode to the REMS interface unit.

Self Test

Prepare a 150 mM solution of KCl. Place the electrode in the solution. Start the Resistance.exe program. Press on the “Take Resistance” button. The resistance indicator should show a resistance between 10 ohms and 100 ohms. A resistance reading out of this range indicates a malfunction.

OPERATING INSTRUCTIONS

After the cell membranes have grown to form a monolayer, the microplate is filled with an electrolyte solution. The REMS KIT electrode probe is then placed in the first cell of the microplate. A command to take a resistance measurement is then sent from the user's program to the REMS KIT program. The REMS KIT software takes a resistance reading and sets a variable indicating that a resistance is available to be read. The user's program retrieves the resistance value. The process is repeated for all cells used in the culture plate.

The precise location of the REMS electrode over each well provides reproducible resistance measurements of tissue grown on porous filter microplates. The use of AC current to measure resistance provides several advantages over DC current measurement including:

1. Absence of offset voltages on measurements.
2. Zero net current is passed through the membrane, therefore it is not adversely affected by a current charge.
3. Electrochemical deposition of electrode metal is avoided.

The REMS Kit Software

The REMs Kit software communicates with the REMS Interface Unit to take resistance measurements and make the measurements available to other Windows applications. The REMs Kit software uses the DDE protocol to communicate with other Windows applications. Two example programs are included with the REMS kit software. They are written in Visual Basic and Labview. The source code provided in these languages can be modified to suit the programmers needs.

Files included on the REMS KIT CD

REMSform.frm This is the Visual Basic Form file for the example program.

Rems.vbp This file is the Visual Basic Project file. It contains the source code for Visual Basic.

rems kit.exe This is the program that performs the actual resistance measurements and communicates with the REMS interface unit and other Windows programs using the DDE protocol. This is the DDE server program.

Resistance.vi This is the LabVIEW example program.

Resistance.exe This is the LabVIEW example program in executable format.

daqdrv This is a driver necessary to run the Rems Kit software. It must be in the same directory as rems kit.exe file.

setup.exe This file is used to install the REMS KIT software and example programs.

LabVIEW runtime engine/Setup.exe This file installs the LabVIEW runtime engine.

The Example Software

Labview 5.0 or higher is required to run the Labview example program. The LabVIEW example executable file is shown above and is named Resistance.exe. Below is a description of controls and indicators used in the Resistance.exe program

Command: This control shows the command that is sent to the rems kit software. The command is generated by the resistance range control. The commands are listed below.

"2K" sets the resistance range to 2000 ohms.

"20K" sets the resistance range to 20 K ohms.

"auto" sets the resistance range to auto select.

Take Resistance: Pressing this button will take a resistance reading.

Device Number: This is the number of the data acquisition card assigned by NIDAQ

Resistance: This indicator shows the Resistance Value retrieved from the REMS KIT server

Resistance Range: This Control selects the Resistance Range. Available ranges are 2000 ohms, 20 K ohms, and auto range. When "Auto range" is selected the best range will be automatically selected.

Exit: This control exits the program and closes the REMSserver program.

The Visual Basic Example Program

Visual Basic 5.0 or higher is required to run the Visual Basic example software. Double-click on REMS.vbp to launch the software. Below is a description of controls and indicators used in the REMS.vbp example.

Connect: Pressing this button launches the REMS KIT.exe server and initializes communications to the server.

Resistance Range: This control selects the Resistance Range. Three resistance ranges are available Auto, 2000 ohms, and 20 K ohms. Auto range will automatically change the resistance range when the other range is more appropriate. The 20 K ohm range selects a resistance range between zero and 20 K ohms. The 2000 ohm range selects a range between zero and 2000 ohms.

Command: This control shows the command that is sent to the rems kit software. The command is generated by the resistance range control. The commands are listed below.

"2K" sets the resistance range to 2000 ohms.
"20K" sets the resistance range to 20 K ohms.
"auto" sets the resistance range to auto select.

Device number: This is the number of the data acquisition board selected by the NI-DAQ software.

Resistance: This indicator shows the resistance returned by the REMS KIT.exe server.

Status: Setting this value to “data read” tells the REMS KIT server to take a resistance reading. Once a resistance value is read this item is set to data available by the REMS KIT.exe program. When quitting the program the value should be set to “exit”.

The DDE interface

The REMS KIT.exe server uses the following variables. These variables need to be specified in the program used to communicate with the REMS KIT.exe server.

Service Name: remsserver

Topic Name: rems

DDE item:	State	This item is set to “data read” by the client program to read a resistance value. It is set to “data available” by the REMS KIT.exe program once a value is read. It is set to “”exit” by the client program to cause REMS KIT.exe to quit.
DDE item:	device	This is the device number of the PCI-6023E
DDE item:	Resistance	This is the resistance value obtained by the REMS KIT.exe program.
DDE item:	command	Available commands are 2K, 20K and auto. “2K” sets the resistance range to 2000 ohms. “20K” sets the resistance range to 20 K ohms. “auto” sets the resistance range to auto select.

THE REMS KIT ELECTRODE

The REMS electrode is designed to handle TEER measurements of cells grown in Falcon HTS Multiwell insert system and Corning Costar HTS Transwell-24 (**REMS KIT**), or Millipore Multiscreen™ Caco-2 96-well microplates (**REMS KITM**).

Each manufacturer's microplates will have different coordinates, and these need to be changed in the software whenever a different plate is used.

Since the electrode is made from soft metal that can be easily damaged when colliding with a hard object, the REMS kit contains a dummy electrode for checking the positioning of the robotic arm. It has the same dimensions as the real electrode with no electrical connection. The two probes on the dummy are made of solid stainless steel and are therefore robust. The user should first mount the dummy electrode on the robot to check the positioning of the robotic arm. The real electrode should not be mounted until the dummy probes can move freely without touching anything but the fluid.

Testing the electrode

The electrode can be tested by measuring the resistance of a blank HTS plate filled with either culture media or 150 mM KCl. The resistance reading of the blank insert is normally in the range of 100 to 300 ohm, depending on the conductivity of solution, the brand of the insert, the type of the tray (96 or 24 well tray) and temperature. This value should be higher than the resistance reading of the electrode without the HTS plate (normally below 100 ohm). If the reading is within this range and is stable and reproducible, the electrode is ready to use.

For Falcon HTS plate user, we offer an optional testing plate that is coated with a conductive polymer that mimics a confluent epithelial membrane's resistance in fluid (WPI's **Syncel HTSF**). However, since the Syncel HTSF cannot be sterilized, it should only be used periodically for maintenance.

MAINTENANCE OF THE ELECTRODE

Cleaning

After using the electrode, it should be rinsed with distilled water and dried with a soft cloth or tissue.

With use, the electrode surface could become coated with foreign materials. This build-up, or contamination, will degrade the performance of the system. If the meter readings become less stable, the electrode can be cleaned as follows:

1. Soak the electrode in 1 N HCl for five minutes.

2. Next soak the electrode in undiluted bleach for five minutes.

CAUTION: Do not let the HCl or bleach come in contact with the stainless steel component of the electrode holder.

3. Rinse the electrode thoroughly with distilled water after this treatment to remove any corrosive fluid remaining.

Sterilization

The electrode is non-sterile as supplied. The electrode cannot be sterilized by autoclaving. Although all of the material used in the electrode can withstand sterilization temperatures, a full evaluation of this procedure on the performance of the electrode has not as yet been completed.The electrode may be sterilized using alcohol (one of the most common methods), ethylene oxide, UV, or a bactericide (*e.g.*, Cidex Plus [WPI **#7364**] or Sporicidin).

A Typical Sterilization Protocol Using Alcohol:

WARNING: Do not leave the electrode in alcohol for more than 30 minutes. Continuously soaking the electrode in alcohol will weaken the protective coating on the electrode and shorten its lifetime.

In a laminar flow hood:

1. Immerse the electrode in 70% ethanol for 15 minutes. Allow it to air dry for 15 seconds.

2. Rinse the electrode in a sterile electrolyte solution similar to the experimental cell culture medium or in 0.1 M KCl or 0.15 M NaCl solution.
3. The electrode is now ready to use.

The electrode can be left in the UV hood to keep it sterile.

NOTE: When the electrode is exposed to strong visible or UV light, a dark colored oxide film will slowly form on the electrode surface. This film normally will not affect the performance of the electrode. To avoid the formation of the film, shield the electrode from strong light.

Storage of the Electrode

The electrode should be rinsed with distilled water and stored dry and in the dark.

TROUBLESHOOTING

Resistance measurements unstable:

Check REMS electrode tip and baseplate electrodes for dirt and film formation. Carefully clean the electrode areas with a cotton swab or a very fine abrasive paper. If electrode is bent, straighten or replace electrode. Operate the system with the REMS electrode in the stabilizing well for a few hours to stabilize measurements.

ACCESSORIES

54230	REMs Kit Electrode
54214	REMS Kit Dummy Electrode
SYNCEL-HTFS	Falcon HTS plate coated with conductive polymer for calibration

SPECIFICATIONS

Membrane Resistance Range ..... 0 to 2000 ohms and 0 to 20 k ohms

AC square wave current ..... +/- 20 micro amps @ 12.5 Hz

Typical measurement time ..... 1 second per measurement.

REMS Interface Unit (RIU) ..... One red and one green LED
Green => RIU On
Red => Sampling

WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

Warning: This equipment is not designed or intended for use on humans.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

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