Applied BioPhysics

CP96 MANUAL

Contents:

The ECIS® CP96 uses microampere, high frequency 48KHz alternating current (AC) to follow the capacitance of gold electrodes used as cell substrates. As a growing monolayer of cells forms upon the electrodes, the capacitance decreases and this information is used to calculate the relative cell coverage (CELL**X**). CELL**X** is reported from 0 to 100 units, where 0 represents a cell-free electrode and 100 represents an electrode covered with a complete confluent layer of cells (a combined cell membrane capacitance of 1 microF/cm2). The complete assay consists of three phases; a zero set phase, a data collection phase and an analytical phase which will yield growth rates. During the zero set phase, the initial capacitance values of the cell-free ECIS electrodes are measured and stored in memory as the zero-point reference. During data collection, the change in capacitance due to the cells is measured and returned as CELL**X**. CELL**X** can be converted to Cell Numbers by a simple calibration procedure.

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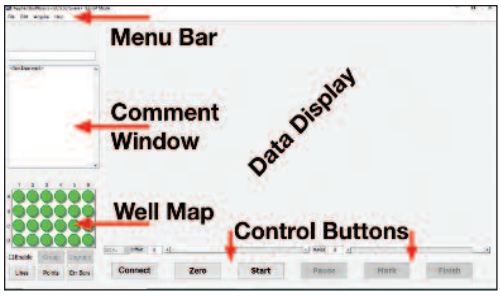
Setting up the CP96:

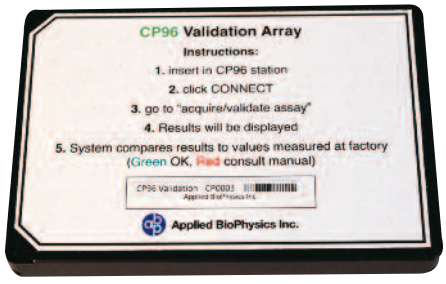
System includes:

- CP96 Station
- Station Controller with power supply
- Laptop PC with CP96 software installed
- CP96 Validation Array
- 2 USB cables
- 6 96W20idf plates

CP96 Controller back

CP96 Station back





CP96 Validation Array

1) System Setup

a. Remove components from packaging.

b. Connect power cable into Station Controller and plug power supply into wall outlet (If there is concern about quality of power, an Uninterruptible Power Supply (UPS) is recommended).

c. Connect laptop to Station Controller by running USB cable from laptop to the **Computer USB** port on Station Controller.

d. Connect Station Controller to CP96 Station by running USB cable from **Unit A** port on back of Station Controller to USB port on back of CP96 Station.

e. Connect power to laptop and turn laptop on to log in.

f. Enter login with username: **ECIS User** (password not necessary).

2) Mount Validation Array to CP96 Station

a. On CP96 Station, slide the two retaining clamps towards outside of station.

b. Insert Validation Array into CP96 Station using correct orientation.

c. Push down on one side of Validation Array and slide retaining clip inward to hold plate down; repeat on opposite side.

3) Start CP96 Software

a. Double-click **CP96-A** Icon and allow load time.

b. Press **Connect** to allow software to recognize attached wells.

c. All 96 wells should appear green in the Well Map. If any wells appear red, reseat Validation Array and repress **Connect**.

d. Repeat until all wells appear green in Well Map.

4) Validate Array

a. Select "Acquire > Validate Assay" from Menu bar.

b. Enter serial number of Validation Array when prompted.

c. Values of calculated "**CellX**" (degree of cell coverage) of the Validation Array will be shown in the display. These are compared to measured values from the Validation Array when the instrument was setup and tested at the factory.

d. Wells will appear green if values are within 2% of expected values.

e. Wells will appear yellow if values are within 2 to 5% of expected values.

f. Wells will appear red if above 5% of expected values.

Running a CP96 experiment (suggested protocol)

1) Incubate CP96 Station

a. Place CP96 Station in supplied poly bag.

b. Store CP96 Station (in bag) in incubator for ~ 1-2 hours.

2) Prepare Well Plate

a. Coat ECIS Cell Proliferation 96 Well plate with matrix proteins (according to protein manufacturers recommendations/protocols).

b. Place 0.2 ml of ABP Electrode-stabilizing Solution (10 mM cysteine in sterile water) to each well.

c. Incubate at room temperature for 1 hour.

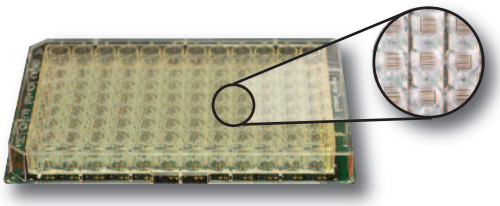
d. Rinse wells twice w/sterile water or saline.

e. Fill wells with 0.2 ml of growth medium without cells.

f. Remove CP96 Station from incubator and from the poly bag.

g. Follow **System Setup** from "Setting Up CP96" protocol if system is not setup.

h. Mount well plate into CP96 Station clamps (Refer to "Setting Up CP96") and place back into incubator.



96W20idf plate

3) Start ECIS CP96 Software

a. Double-click **CP96-A** icon to open software.

b. Press **Connect** to test connection of each well (shown on Well Map).

- All wells with medium should appear green; wells without medium or are not connected will appear red.
- If necessary, reseat well plate and retest connection until desired wells are green.

c. Once CP96 well assembly has reached equilibrium within incubator conditions, press **Zero** to set the zero reference. A popup window will allow two options:

- **Load Dataset** : zero reference is set from previous datafile that you will select and open.
- **Zero** : a new zero reference is measured and stored in new file.

d. A diagram of the wells and measured capacitance of cell-free electrodes will be displayed.

- Wells with capacitance values > 75 nF are desired for open electrodes & are marked green.
- Wells with capacitance values < 75 nF are marked orange.

e. Once zero reference is set, **Start** button will become available to begin running experiments.

4) Running Experiments

a. Remove well plate from CP96 Station.

b. Under aseptic conditions, add cells (7,500/well recommended) to each well in a final volume of 0.3 ml.

c. Return well plate w/cells to CP96 Station inside incubator.

d. Press **Check** to verify connections.

e. Select cell-free wells on Well Map; a white center will be shown.

f. Press **Start** to begin experiment.

g. Results will show on diagram using **CellX** (degree of cell coverage).

h. During experiment **Pause** may be pressed to pause the experiment for cell treatment, etc.

- After placing well plate back into CP96 Station, press **Check** to verify connection.
- Press **Resume** to continue experiment.

i. To end experiment, press **Finish** .

Commands

Menu bar commands

FILE

Open	Loads a previous experiment.
Recent Files	Loads a recent experiment.
Export Graph	Exports the current graph in a figure format (jpg, tif, png, …).
Export CELLX	Exports the current experiment to a csv. file.
Close	Close the current experiment.
Exit	Ends the program.

EDIT

Color Palette	Select the well color scheme.
Group Map	Select the well grouping scheme.
Error Bars	Adjust the error bar size.

ACQUIRE

Setup New Expt.	Resets software to run a new experiment.
Activate All Wells	Overrides well check to activate all wells.
Find Instrument	Set ECIS COM port and look for instrument.
Validate Assay	Selects the Validation mode of the instrument.
Growth Rate	Calculate growth rates.

HELP

Manual	Open the HTML manual.
Open Log File	Opens the serial log file for inspection.
About	Gives software version and author.

Helpful Tips:

1. It is very important to minimize temperature changes during the initial setup and medium exchanges. Insure all solutions are pre-warmed to 37°C.
2. Before setting the zero-point reference make sure the medium in the CP96 well assembly is at incubator temperatures. Cold medium may cause the zero-point reference to be set too high leading to negative CP96 values.
3. When changing medium in a tissue culture hood use a warming plate to keep the CP96 well assembly at 37°C.