For life science research only. 
Not for use in diagnostic procedures.
The iCELLigence System

The iCELLigence™ System is a microelectronic biosensor system for cell-based assays, providing dynamic, real-time, label-free cellular analysis for a variety of research applications in drug development, toxicology, cancer, medical microbiology, and virology. This Real-Time Cellular Analysis (RTCA) technology allows researchers to increase productivity and exceed the limits of endpoint analysis by capturing data throughout the entire time course of an experiment and obtaining more physiologically relevant data.

Impedance-Based Technology

The presence of cells on top of the electrodes will affect the local ionic environment at the electrode/solution interface, leading to an increase in the electrode impedance. An increase in the number of cells attached on the electrodes leads to an increase in electrode impedance. In addition, the impedance value reflects the quality of the cell interaction with the electrodes. For example, increased cell adhesion or spreading will lead to an increase in electrode impedance. Thus, electrode impedance, which is displayed as Cell Index (CI) values, can be used to monitor cell viability, number, morphology, and extent of adhesion in a number of cell-based assays. In addition, the electronic signal used is very weak and non-invasive to the live cells.

Wireless Data Connection

Conveniently view experimental data at your desk

- Experimental data is automatically synchronized to the Control Unit once it wirelessly connects to the iCELLigence instrument
- Share experimental data (.png and .csv format) wirelessly via email


The iCELLigence System consists of two components: the iCELLigence Control Unit and the iCELLigence Instrument with two integrated plate cradles for measuring cell responses in parallel.
Simple Workflow
No cell labeling required, fully automated, physiological conditions

User-Friendly RTCA Software
Pre-defined protocols guide you through experimental set-up and analysis in seconds

E-Plate L8: Cellular Assay in an 8-Well Format
Features large microtiter wells with view area for complementary assays

- Compatible with multichannel pipettes
- Sufficient sample quantity for protein and nucleic acid extractions
- Visualize changes using a microscope

Compatibility with multichannel pipettes:
- Phospho-Histone3
- β-actin

Western blot using cell lysates harvested from E-Plate L8. A549 cells (40,000/well) were treated with Paclitaxel or vehicle control (DMSO) for 24 hours. An average total of 160μg of protein were harvested from untreated cells in each E-Plate L8 well. Lysates containing 20μg of protein were loaded in each well of SDS-PAGE.

The spacing of the wells is 9 mm center-to-center as per the ANSI/SBS 4-2004 standard for 96-well microtiter plates.

E-Plate VIEW area enables cell visualization by microscopy as well as measuring cell responses. H460 cells (31,500 cells/well) were stained by crystal violet and observed under bright-field microscopy at 10x magnification.
Application Highlights

**Cell Adhesion and Spreading**

- Real-time monitoring of cell adhesion and spreading.
- Label-free assay requires no fixation, staining or any other sample processing.
- Easy quantification of the kinetics of adhesion and spreading.
- Rapid optimization of cell density and extracellular matrix coating conditions.

**Functional Monitoring of GPCR Signaling**

- Assay endogenous GPCRs with primary cells, stem cells, and/or other disease relevant cells.
- Simultaneous screening for GPCR function across all coupling classes: $G_\alpha$, $G_\beta$, $G_\gamma$, $G_{12/13}$.
- Detection of traditionally difficult classes: $G_i$ and $G_{12/13}$.
- Detection of functional selectivity.
- De-orphaning GPCRs.

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**Figure 1.** (A) Dynamic monitoring of cell attachment and spreading of A549 cells plated on titrated Collagen IV coated surfaces. (B) The Cell Index correlates with the extent of cell numbers observed with brightfield imaging analysis. (C) Summary table shows cell adhesion and spreading (Max CI), time needed to achieve 50% adhesion and spreading (TR (CI, 50%)), and the corresponding cell adhesion rate (SR CI).

**Figure 2.** Pharmacological study of endogenous histamine GPCR function. (A) HeLa cells were seeded at 24,000 cells per well on E-Plates L8, treated the next day with increasing concentrations of histamine, and the cell response was monitored every 30 seconds for 30 minutes. (B) Plotting the peak cell index responses versus the corresponding log concentrations allows calculation of the EC50 of histamine.
Virus-Mediated Cytopathogenicity

- A simple alternative method to the plaque test for measuring the lytic activity of viruses.
- Provide quantitative information about the onset and kinetics of viral mediated cytopathic effects (CPE).
- Rapidly identify the optimal viral titer and assay time point for subsequent screening of inhibitory compounds, neutralizing antibodies and neutralizing serums.

**Figure 3. Dynamic monitoring of HEK 293 cells during viral infection.** (A) Normalized Cell Index values of growing cells, (B) Normalized Cell Index values of confluent cells. The virus-mediated effect on adhesion, spreading, and proliferation of the cells was dynamically monitored every 15 minutes. Time of addition of virus at 68.5 hours is indicated by the black vertical line, the time point when the Cell Index had decreased to 50% of the maximum Cell Index value (CI_{50}) is indicated by the dotted orange lines.

**Note:** The time it takes to reach CI_{50} is defined at CIT_{50}, which is indicated by the orange arrows. CIT_{50} has been shown to linearly correlate with virus titer (log10 PFU) (Journal of Virological Methods (2011, 173 (2), 251-8)).

Compound-Mediated Cytotoxicity

- Real-time data allows identification of the optimal times for treatment and data collection.
- Non-invasive assay is performed in a tissue culture incubator, allowing for analysis by standard viability assays.

**Figure 4. Dynamic monitoring of compounds mediating cytotoxicity and apoptosis.** (A) HeLa cells were treated with MG-132 (1 µM) and 5-FU (100 µM), and Cell Index was continuously monitored for 64 hours. At 16 hours (B) and 64 hours (C) post compound addition, apoptosis assays were conducted with the cells harvested from the E-Plate L8. In comparison to the untreated control, fold increases of apoptosis induction were shown for both compounds.
Cell-Mediated & Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

- Real-time monitoring of cell-mediated cytotoxicity & ADCC.
- Direct, sensitive, and specific measurement of target cell changes.
- A homogenous assay for easy quantification of the kinetics of the cytotoxic response, both short and long-term.
- Rapid optimization of cell density and effector/target ratios.

Figure 5. Real-time, label-free monitoring of NK92 cell-mediated cytolysis of DU145 cells.
DU145 cells were seeded into an E-Plate and monitored every 15 minutes. At 20.5 hours, NK92 cells were added to the wells at E:T ratios ranging from 0.47:1 to 30:1. The effect of NK cell addition was monitored for 67 hours. (A) This plot shows data normalized to the last time point before NK cell addition. (B) Same data as above, but with non-treated control cells (red line) defined as baseline. Other curves are plotted in relation to this baseline.

Figure 6. Effect of the anti-IGF-1R antibody on NK cell-mediated cytolysis of DU145 cells (E:T ratio = 3.75:1). DU145 cells were seeded into an E-Plate and monitored every 15 minutes. After 20.5 hours, NK92 cells were added to the wells at an E:T ratio of 3.75:1. Target cells were pre-incubated in advance with different concentrations of anti-IGF-1R antibody for 30 minutes. The effect of NK cell and anti-IGF-1R addition was monitored for 67 hours.

(A) Plot of normalized CI values of the entire 88 hours of the experiment. Data were normalized to the last time point before NK cell addition and curves were plotted with control wells (DU145 cells only) set as baseline. (B) Calculation of IC_{50} after 60 hours. (C) Calculation of time-dependent IC_{50} from 9 hours after NK cell addition to the end of the experiment at 88 hours.
Application Highlights continued

Cell Barrier Function

- A label-free alternative to solute permeability and transendothelial electrical resistance (TEER) assays.
- Real-time assay is conducted under normal tissue culture conditions, allowing for monitoring of barrier function disruption as well as recovery.
- Non-invasive nature of the readout allows for orthogonal assays conducted on the same device, including visual monitoring of cell density by microscopy.

Ordering Information

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** Figure 7. The protective effect of Activated Protein C (APC) on endothelial barrier function assessed by solute permeability assays and RTCA platform. Confluent EA.hy926 cells were pre-incubated with APC, followed by addition of thrombin to induce endothelial permeability. (A) The cell barrier dysfunction induced by 10 nM thrombin was assessed by measuring Evans blue permeability. Cells were incubated with 20 nM APC for 4 hours prior to thrombin treatment. (B) The cell barrier dysfunction induced by 10 nM thrombin was assessed by measuring FITC permeability. Cells were incubated with 90 nM APC for 2 hours prior to thrombin treatment. (C) Dynamic impedance-based monitoring of thrombin (2 nM) induced endothelial barrier dysfunction. Cells were incubated with 25 nM APC for 4 hours prior to thrombin treatment. The plot shows data normalized to the last time point before thrombin addition, and curves were plotted with control wells set as baseline. (D) The maximal change in the endothelial barrier function as percentage of the control derived from Cell Index changes. Asterisk denotes a statistically significant difference (p<0.05).

** Data and figures courtesy of F. Stavenuiter, E. Bouwens, R. Sinha, L. Mosnier, J.H. Griffin, Dept. of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA.
Product Specifications

RTCA iCELLigence Instrument Specifications

**Package Includes:** RTCA iCELLigence Instrument, RTCA iCELLigence USB Cable, USB Power Adapter (N. American), International USB Power Adapter, RTCA iCELLigence Tool Set (including Pliers, RTCA iCELLigence Contact Pins (10x), RTCA Contact Pin Insertion Tool, Resistor Plate L8 (2x), RTCA iCELLigence S/N Label)

**Dimensions:** 18.8 cm × 20.0 cm × 16.9 cm (W × D × H) (fully opened)

**Weight:** 1.9 kg

**Electrical Input:** +5 VDC, 1 W max

**Test Signal:** 22 mV rms ± 20% with max. 5 mV DC offset at 10 kHz

**Impedance Measurement Accuracy:** ± (1.5% + 1 Ω)

**Impedance Measurement Repeatability:** 0.8%

**Impedance Dynamic Range:** 10 Ω to 5 kΩ

**Communication:** Compatible with IEEE 802.11 b/g/n

**Environment:** Temperature, +20°C to +40°C; relative humidity, 98% maximum without condensation

RTCA iCELLigence Control Unit Specifications

**Package Includes:** Tablet with Pre-installed RTCA iCELLigence Software, Charge Cable, USB Power Adaptor (N. American)

≥16 GB Storage

≥ 40 Watt-Hour Rechargeable Battery

**Communication:** Wi-Fi (IEEE 802.11 b/g/n)

E-Plate L8 & E-Plate L8 PET* Specifications

**Dimension:** 4.0 cm × 8.7 cm × 1.9 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 microtiter plates

**Volume:** 830 µl ± 10 µl (654 µl ± 10 µl recommended for experiments)

**Bottom Area:** 64 mm² ± 10%

**Electrical Interface:** Interface with iCELLigence Instrument

**Sensor Impedance:** 5.6 Ω ± 1.7 Ω at 10 kHz, when measured with a 1X PBS Solution

**Material:** Biocompatible surfaces; UV irradiated

**Environment:** Temperature, +20°C to +40°C; relative humidity, 98% maximum without condensation

*PET = Polyethylene Terephthalate

Learn more about the enabling technology of the iCELLigence System and its broad range of applications at www.aceabio.com

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