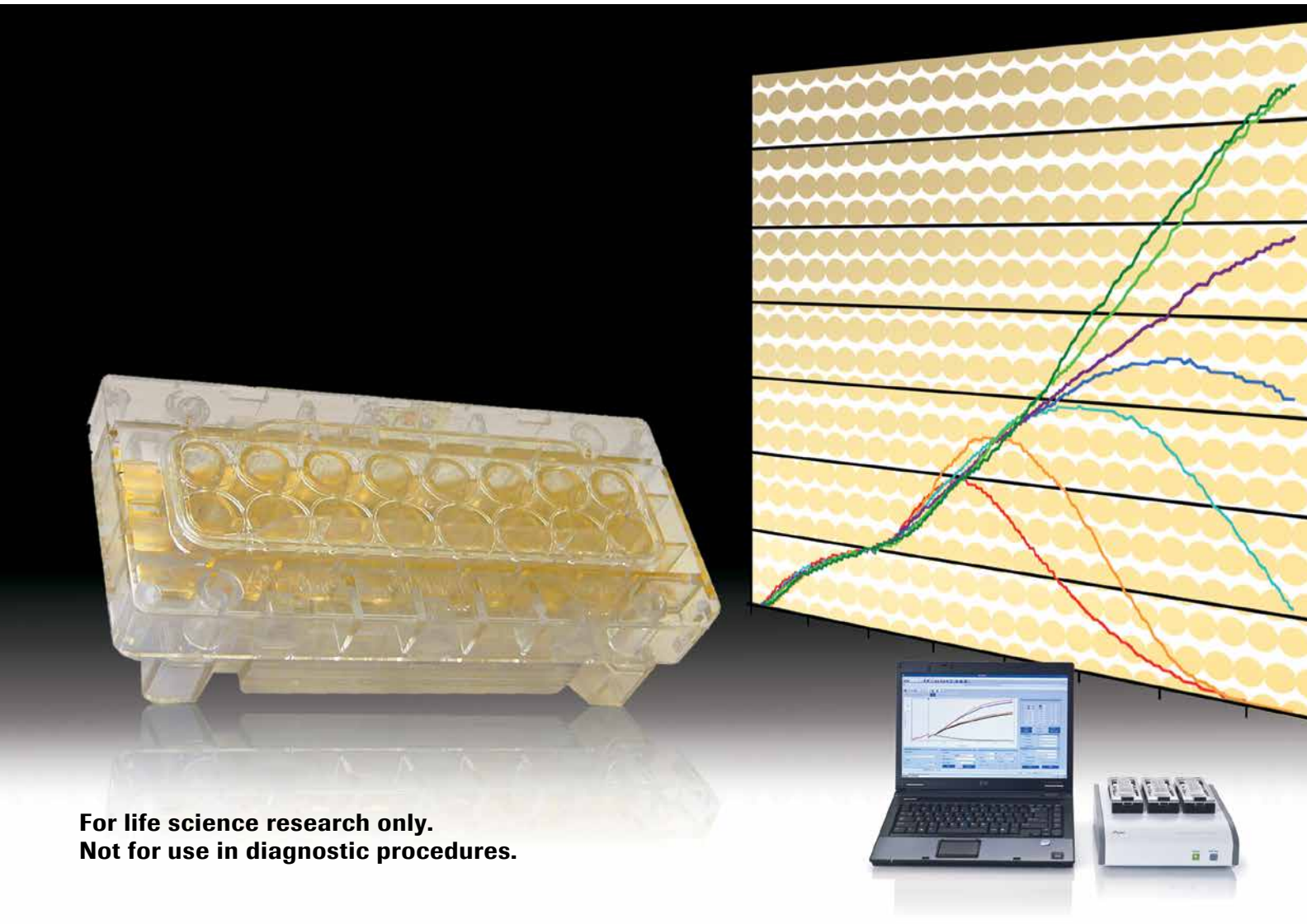
ACEA Biosciences, Inc.

xCELLigence

xCELLigence RTCA DP Instrument

Flexible Real-Time Cell Monitoring



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

The xCELLigence RTCA DP Instrument

Flexible Real-Time Cell Monitoring

The RTCA DP Instrument expands the throughput and application options of the xCELLigence Real-Time Cell Analyzer (RTCA) portfolio. Featuring a dual-plate (DP) format, the instrument measures impedance-based signals in both cellular and cell invasion/migration (CIM) assays – without the use of exogenous labels. With outstanding application flexibility, the RTCA DP Instrument supports multiple users performing short-term and long-term experiments.

Explore the wide range of applications

- **Cell invasion and migration assays**
- **Compound- and cell-mediated cytotoxicity**
- **Cell adhesion and cell spreading**
- **Cell proliferation and cell differentiation**
- **Receptor-mediated signaling**
- **Virus-mediated cytopathogenicity**
- **Continuous quality control of cells**

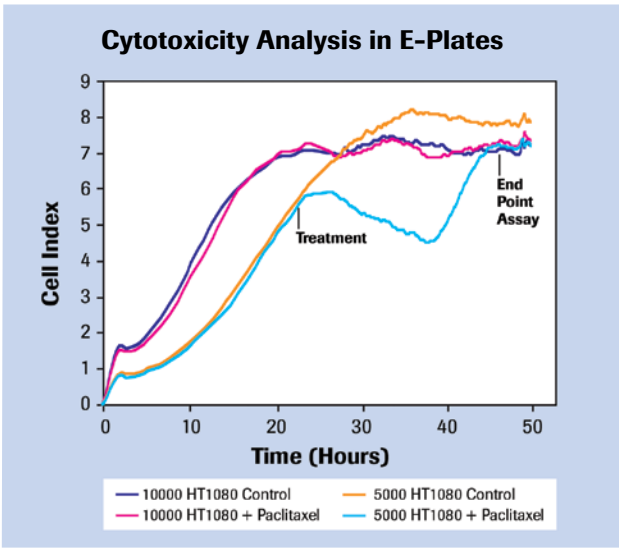


Figure 1: Reveal cytotoxic effects through continuous monitoring. HT1080 cells were seeded in an E-Plate at two different densities (5,000 and 10,000 cells) and treated 24 hours later with 12.5 nM Paclitaxel, or DMSO as a control. As shown by the Cell Index profile, which reflects cell adherence, the antimitotic effect of Paclitaxel was observed in HT1080 cells that were proliferating, whereas confluent cells showed no response.

The xCELLigence System continuously and non-invasively detects cell responses throughout an experiment, without the use of exogenous labels that can disrupt the natural cell environment.

- **Obtain complete, continuous data profiles** from cell responses generated during *in vitro* experiments (Figure 1).
- **Take advantage of real-time data** to identify optimal time points for downstream assays.
- **Combine real-time monitoring of cellular responses with complementary functional endpoint assays**, and maximize data quality before, during, and after your experiment.

RTCA Control Unit **RTCA DP Analyzer**

Compact. Convenient. Versatile.

The RTCA DP Instrument consists of two components: the RTCA Control Unit and the RTCA DP Analyzer with three integrated stations for measuring cell responses in parallel or independently.

- Choose from three types of impedance-based 16-well plates:
  - E-Plate 16 and E-Plate VIEW 16 for cellular assays
  - CIM-PLATE 16 for cell invasion/migration assays
- Use all three different plate types in any combination.
- Easily achieve optimal cell culture conditions by placing the RTCA DP Analyzer and plates into standard CO_2 incubators.

E-Plates for the RTCA DP Instrument

More Flexibility. More Data. More Insight.

Obtain detailed information about your cells with the versatile RTCA DP Instrument, which supports up to three plates of any type – E-Plate 16, E-Plate VIEW 16, or CIM-Plate 16 – in any combination. For example, cell invasion/migration assays and cytotoxicity assays or short- and long-term assays may be run simultaneously.

E-Plate 16 and E-Plate VIEW 16: Cellular Assays in a 16-Well Format

- Quantitatively monitor changes in cell number, cell adhesion, cell viability, and cell morphology.
- Easily add compounds during an experiment.
- Assess short- and long-term cellular effects.
- With the E-Plate VIEW 16, observe measured changes using microscopes.

E-Plate 16

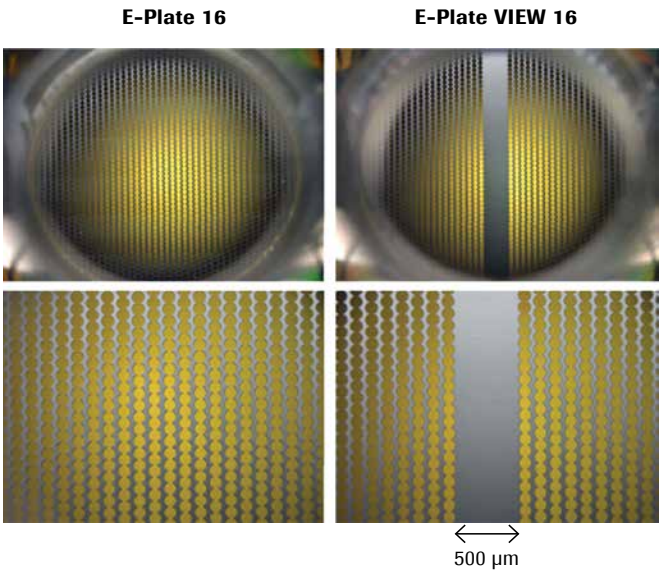
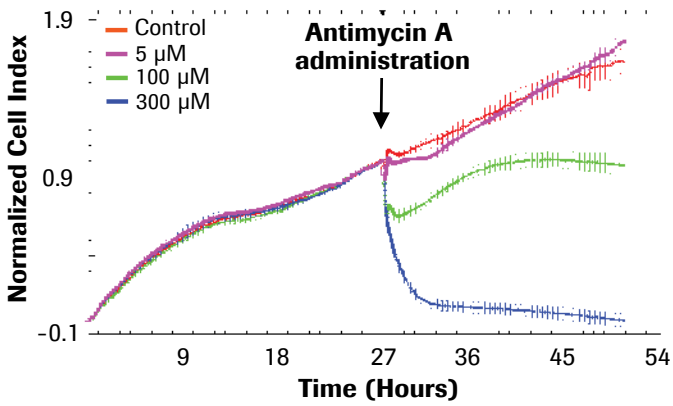


Figure 2: Easily visualize cells while measuring cell response with xCELLigence System E-Plate VIEW technology. A modified version of the standard E-Plate 16, the E-Plate VIEW 16 enables image acquisition using microscopes or automated cell-imaging systems. For the modification, four rows of microelectrode sensors were removed in each well to create a window for visualizing cells. Approximately 70% of each well bottom is covered by the microelectrodes, providing cell impedance measurements nearly identical to those obtained with the standard E-Plate 16. Both plate types can be used in parallel.



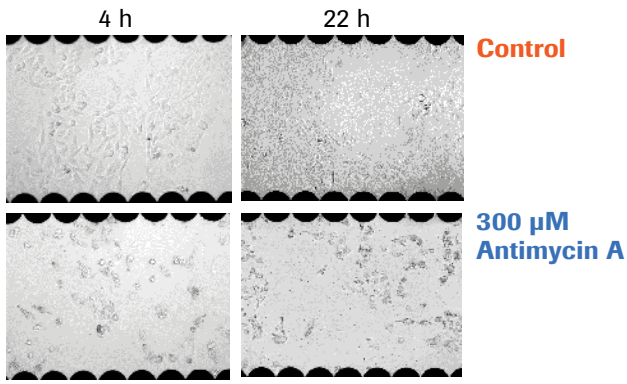


Figure 3: Continuously monitor cells and determine optimal time points for assessing cytotoxicity. Cell proliferation and cell death were continuously monitored using the xCELLigence RTCA DP Instrument. The optimal time points for visual inspection of HeLa cells were determined and images taken 4 and 22 hours after compound treatment using a Z16 Apo Microscope with light base (Leica Microsystems).

CIM-Plate 16:
Quantitative Cell Invasion/Migration Analysis

- Monitor cell invasion and migration continuously in real time over the entire time course of an experiment.
- Eliminate time-consuming manual detection (Figure 4).
- Perform CIM analysis in a convenient one-well system (Figure 5).

CIM-Plate 16

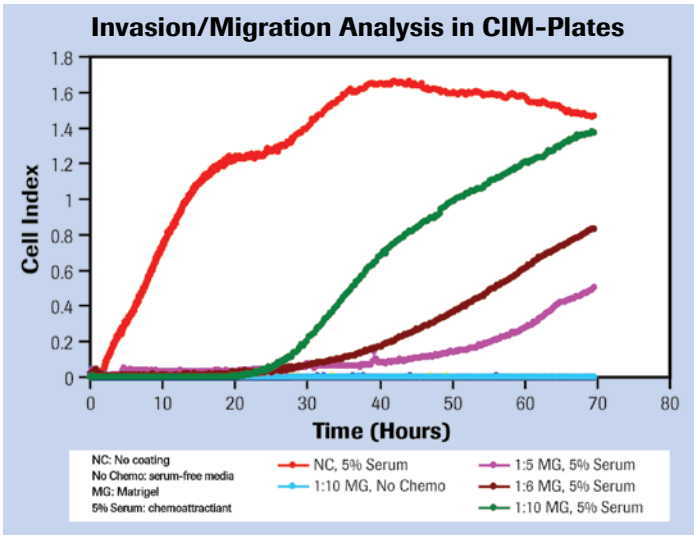


Figure 4: Quantitatively measure the rate and onset of invasion while concurrently assessing migration. HT1080 cells (2 x 10^4) were seeded in the upper chamber of CIM-Plate wells coated with varying dilutions of Matrigel, or in wells with no coating. Serum was added to the lower chamber of selected wells as a chemoattractant. Invasion was observed and migration monitored continuously over a 70-hour period. All serum-starved samples resulted in base-line Cell Index levels, indicating the absence of invasion/migration, while those wells with chemoattractant induced migration.

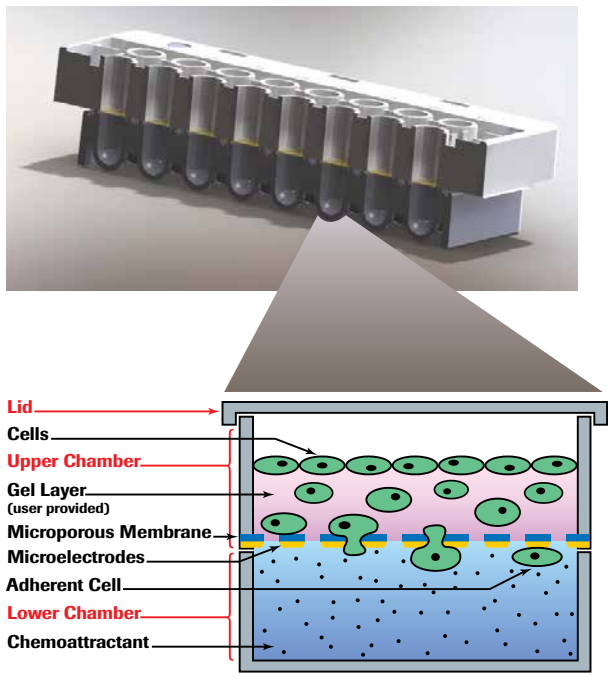


Figure 5: Analyze invasion/migration in real time with the CIM-Plate 16. The plate features two separable sections for ease of experimental setup. Cells seeded in the upper chamber move through the microporous membrane into the lower chamber that contains a chemoattractant. Cells adhering to the microelectrode sensors lead to an increase in impedance, which is measured in real time by the RTCA DP Instrument.

E-Plate Insert 16: Co-Culture in Real-Time

- Continuously monitor indirect cell-cell interactions.
- Assess short- and long-term cell response without labor-intensive labeling and microscopy.
- Co-culture different cell typtes under physiological conditions for a broad range of applications, including:

  Cancer Research: Assess paracrine stimulation of cancer cell proliferation by fibroblasts.

  Immunology: Investigate immune cell interactions.

  Stem Cell Research: Monitor proliferation and differentiation in the presence of stimulation cells.

  Toxicology: Determine cytotoxicity of agents and assess effects of cytokine release.

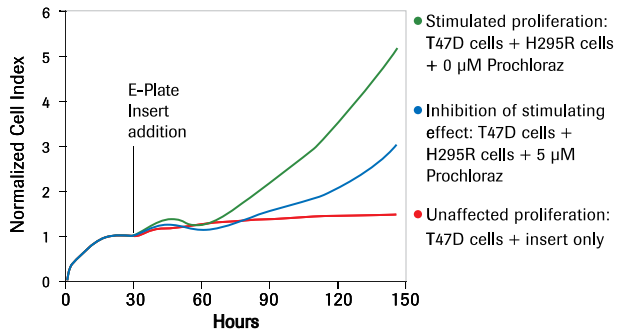
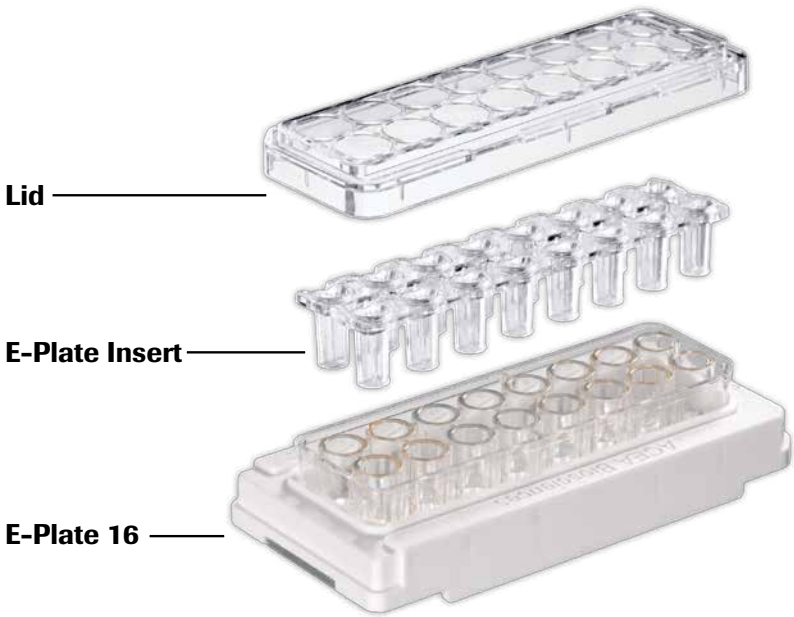


Figure 6. Real-time monitoring of co-culture-induced proliferation stimulation and its inhibition using the E-Plate Insert. Intercellular interactions play an important role in normal cell development and tumorigenesis. Results show that the proliferation of hormone-responsive tumor cells is likely mediated by hormones and growth factors exchanged between the two cell populations separated by the E-Plate Insert.

Elevated T47D cell proliferation on the E-Plate (green trace ■) was induced by hormone secretion of H295R cells in the insert, and inhibited by the hormone synthesis inhibitor Prochloraz (blue trace ■). Incubation of T47D cells with only the E-Plate Insert did not affect proliferation (red trace ■).



Selected Publications for the RTCA DP Instrument

1. Cell Invasion and Migration

MicroRNA-200c Represses Migration and Invasion of Breast Cancer Cells by Targeting Actin-Regulatory Proteins FHOD1 and PPM1Ferences.
Jurmeister S, Baumann M, Balwierz A, Keklikoglou I, Ward A, Uhlmann S, Zhang JD, Wiemann S, Sahin O.
Mol Cell Biol. 2012; 32(3):633–651.

c-Myb regulates matrix metalloproteinases 1/9, and cathepsin D: implications for matrix-dependent breast cancer cell invasion and metastasis.
Knopfová L, Beneš P, Pekarčíková L, Hermanová M, Masařík M, Pernicová Z, Souček K, Smarda J.
Mol Cancer. 2012; 11:15.

Comparative Analysis of Dynamic Cell Viability, Migration and Invasion Assessments by Novel Real-Time Technology and Classic Endpoint Assays.
Limame R, Wouters A, Pauwels B, Fransen E, Peeters M, Lardon F, De Wever O, Pauwels P.
PLoS One. 2012; 7(10): e46536.

2. Compound-mediated Cytotoxicity/Apoptosis

Screening and identification of small molecule compounds perturbing mitosis using time-dependent cellular response profiles.
Ke N, Xi B, Ye P, Xu W, Zheng M, Mao L, Wu MJ, Zhu J, Wu J, Zhang W, Zhang J, Irelan J, Wang X, Xu X, Abassi YA.
Anal Chem. 2010; 82(15):6495-503.

Kinetic cell-based morphological screening: prediction of mechanism of compound action and off-target effects.
Abassi YA, Xi B, Zhang W, Ye P, Kirstein SL, Gaylord MR, Feinstein SC, Wang X, Xu X.
Chem Biol. 2009; 16(7):712-23.

3. Cell-mediated Cytotoxicity

Real-time profiling of NK cell killing of human astrocytes using xCELLigence technology.
Moodley K, Angel CE, Glass M, Graham ES.
J Neurosci Methods. 2011; 200(2): 173-180.

Unique functional status of natural killer cells in metastatic stage IV melanoma patients and its modulation by chemotherapy.
Fregni G, Perier A, Pittari G, Jacobelli S, Sastre X, Gervois N, Allard M, Bercovici N, Avril MF, Caignard A.
Clin Cancer Res. 2011; 17(9): 2628–37.

4. Cell Adhesion and Cell Spreading

A role for adhesion and degranulation-promoting adapter protein in collagen-induced platelet activation mediated via integrin a2b1.
Jarvis GE, Bihan D, Hamaia S, Pugh N, Ghevaert CJ, Pearce AC, Hughes CE, Watson SP, Ware J, Rudd CE, Farndale RW.
Journal of Thromb Haemost. 2012; 10(2): 268–277.

Dynamic monitoring of cell adhesion and spreading on microelectronic sensor arrays.
Atienza JM, Zhu J, Wang X, Xu X, Abassi Y.
J Biomol Screen. 2005; 10(8): 795-805.

Selected Publications continued

5. Receptor-mediated Signaling

Impedance responses reveal b2-adrenergic receptor signaling pluridimensionality and allow classification of ligands with distinct signaling profiles.
Stallaert W, Dorn JF, van der Westhuizen E, Audet M, Bouvier M.
PLoS One. 2012; 7(1): e29420.

Label-free impedance responses of endogenous and synthetic chemokine receptor CXCR3 agonists correlate with Gi-protein pathway activation.
Watts AO, Scholten DJ, Heitman LH, Vischer HF, Leurs R.
Biochem Biophys Res Commun. 2012; 419(2):412-8.

Impedance measurement: A new method to detect ligand-biased receptor signaling.
Kammermann M, Denelavas A, Imbach A, Grether U, Dehmlow H, Apfel CM, Hertel C.
B*iochem Biophys Res Commun*. 2011; 412(3): 419-424.

6. Virus-mediated Cytopathogenicity

Novel, real-time cell analysis for measuring viral cytopathogenesis and the efficacy of neutralizing antibodies to the 2009 influenza A (H1N1) virus.
Tian D, Zhang W, He J, Liu Y, Song Z, Zhou Z, Zheng M, Hu Y.
PloS One. 2012; 7(2):e31965.

Real-time monitoring of flavivirus induced cytopathogenesis using cell electric impedance technology.
Fang Y, Ye P, Wang X, Xu X, Reisen W.
J Virol Methods. 2011; 173(2):251–8.

7. Quality of Control of Cells

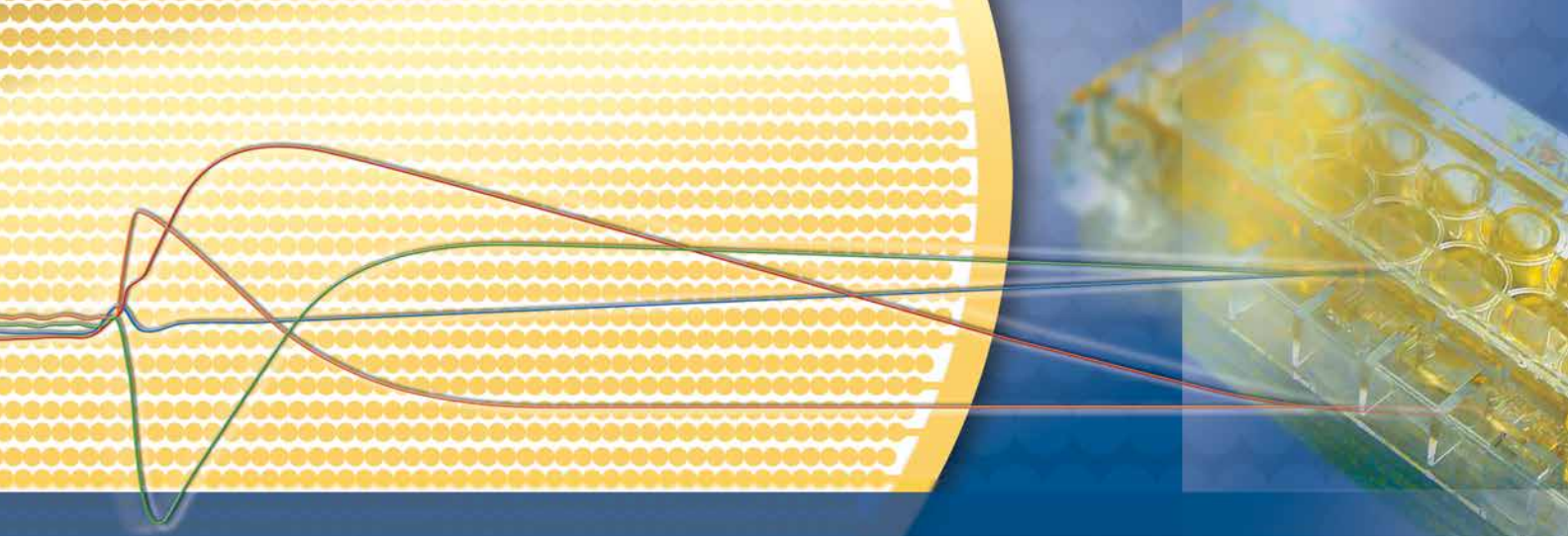
Rapid and quantitative assessment of cell quality, identity, and functionality for cell-based assays using real-time cellular analysis.
Irelan JT, Wu MJ, Morgan J, Ke N, Xi B, Wang X, Xu X, Abassi YA.
J Biomol Screen. 2011; 16(3):313-22.

Live cell quality control and utility of real-time cell electronic sensing for assay development.
Kirstein SL, Atienza JM, Xi B, Zhu J, Yu N, Wang X, Xu X, Abassi YA.
Assay Drug Dev Technol. 2006; 4(5):545-53.

8. Endothelial Barrier Function

An inverted blood-brain barrier model that permits interactions between glia and inflammatory stimuli.
Sansing HA, Renner NA, MacLean AG.
J Neurosci Methods. 2012; 207(1):91–6.

A dynamic real-time method for monitoring epithelial barrier function in vitro.
Sun M, Fu H, Cheng H, Cao Q, Zhao Y, Mou X, Zhang X, Liu X, Ke Y.
Anal Biochem. 2012; 425(2):96–103.



Ordering Information for xCELLigence RTCA DP System

Product	Cat. No.	Pack Size
xCELLigence RTCA DP Instrument	**00380601050**	**1 Bundled Package**
RTCA DP Analyzer	05469759001	1 Instrument
RTCA Control Unit	05454417001	1 Notebook PC
E-Plate 16	05469830001	6 Plates
	05469813001	6 x 6 Plates
E-Plate VIEW 16	06324738001	6 Plates
	06324746001	6 x 6 Plates
E-Plate Insert 16	06465382001	1 x 6 Devices (6 16-Well Inserts)
CIM-Plate 16	05665817001	6 Plates
	05665825001	6 x 6 Plates
CIM-Plate 16, Assembly Tool	05665841001	1 Assembly Tool

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

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

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