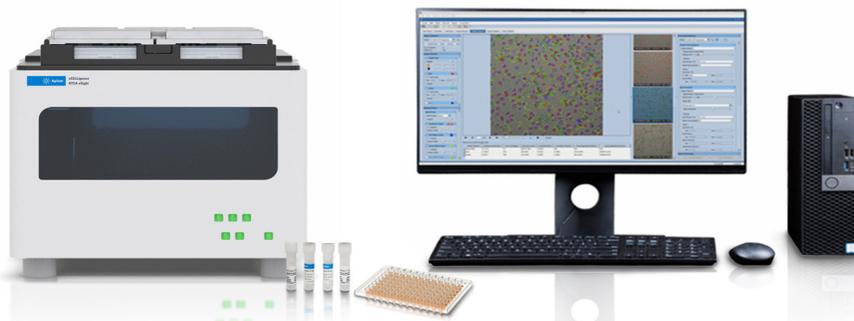


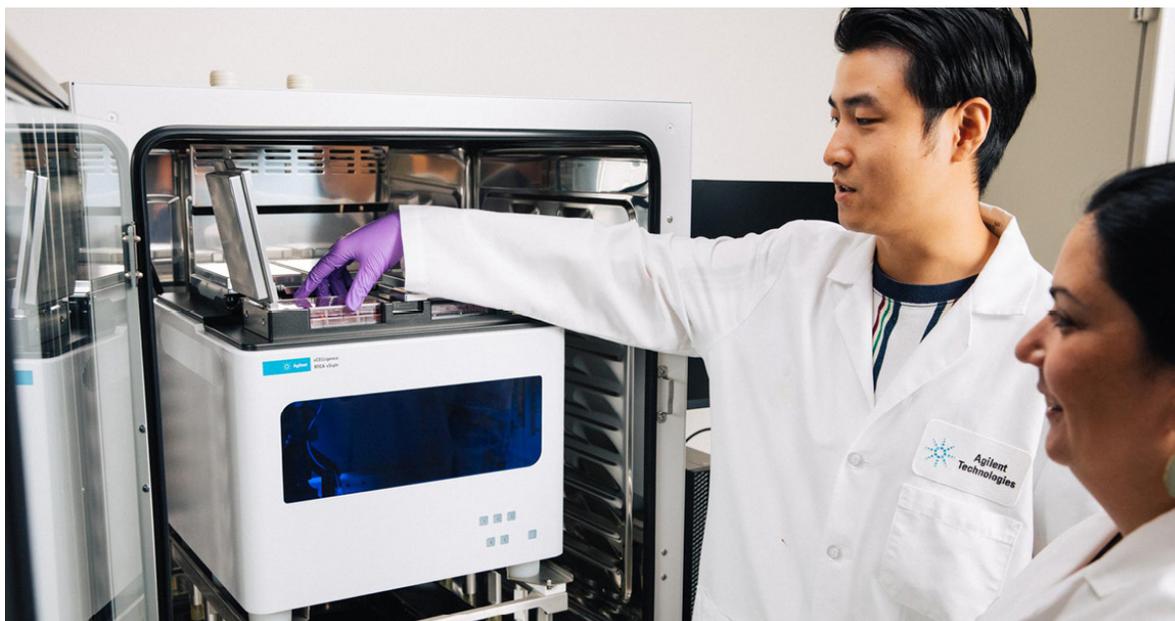
Measure Cell Movement, Health, and Function With xCELLigence RTCA eSight

Multiparametric real-time automated readouts using
simultaneous cellular impedance and live cell imaging



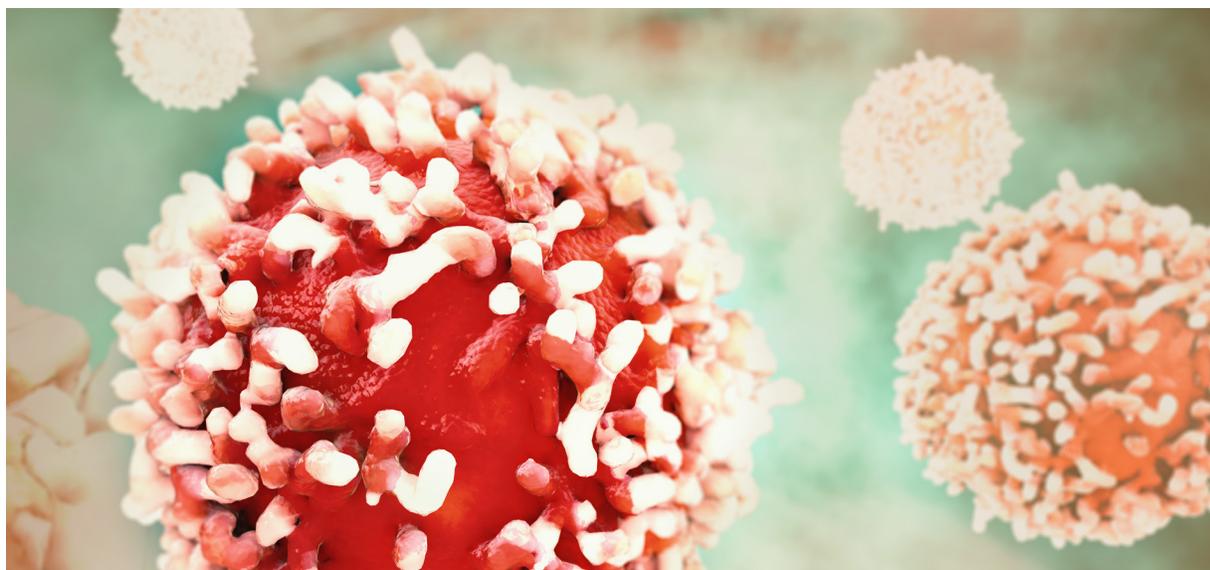
Get Two Instruments and Twice the Results from One Powerful System

The Agilent xCELLigence RTCA eSight is currently the only platform on the market capable of collecting live-cell imaging and real-time impedance data at the same time.



Unlock the true potential of your immuno-oncology and drug discovery research by understanding crucial biological insights and changes in your cells in real-time. The xCELLigence RTCA eSight measures and monitors the health and viability of a cell population to fully understand the effects of a new drug, cell or gene therapy.

What are the advantages of live-cell analysis?



- **Maintain the integrity of your experiments and the cellular environment by analyzing viable, living cells.**
Traditional assays quickly shift from live-cell to endpoint, destroying the cells. With the RTCA eSight platform, cells are measured in a highly physiologically relevant environment. Also, each of the five cradles is independent of one another, ensuring that you can add or change plates without disturbing cells in the other cradles.
- **Get information-rich analysis by multiple readouts and the power of dual technology.**
Why rely on imaging alone, when you can quickly start your experiments with the ease of impedance? Impedance requires minimal hands-on time and software training. Together, impedance and imaging provide deeper insights to understand biological phenomena and hone in on drug mechanism of action. Generate impedance graphs, images, and movies with ease.
- **Start your kinetic analysis in record time to get answers quickly.**
Live-cell kinetic analysis has never been easier than with the eSight. Don't rely on just a single time or end point. Continuous analysis ensures you capture biological events, responses, or interactions as they happen. 96-well formats enable quick optimization and high throughput capability for rapid QC, seeding densities, or concentrations.
- **Easily achieve live-cell analysis with a simple workflow using the RTCA eSight.**
Analyze cells in the incubator for days or weeks without disturbing your cells. Each cradle provides the best user experience allowing for simple workflow, scheduling, and accessibility. Drive and improve experiment outcomes whether you are enhancing cell culture conditions or diving into complex cell-cell interactions.

Advance Your Cell Health Research with One Simple, Efficient Workflow

Maximum Efficiency with Multiplexing

- **Multiplex readouts:** Simple mix and read reagents or label-free (impedance)
 - **Built-in validation:** Use the power of both impedance and imaging to verify your results
 - **Reproducible:** Generate data while minimizing costs and time when doing repeat experiments
 - **High throughput:** Run 5x96-well plates simultaneously
 - **Flexible:** Automatically quantify treatments, generate dose-response curves, or determine mechanism of cell death
-

Gain More Insights

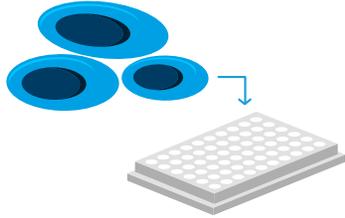
- **Expanded biological insights:** Understand biological phenomena, and be more confident in your experiments with two different readouts.
 - **More physiologically relevant:** Capture precious cellular and biological events as they happen, avoid missing crucial time points seen with traditional end point assays
 - **Insightful kinetic readouts:** Measure short or long term effects, from seconds to days
 - **Sensitive:** Capture key cellular events with the xCELLigence impedance biosensor technology
-

Simple Workflow

- **Hands-off:** Run assays without removing cells from the incubator
- **Automated:** Acquires and analyzes impedance data and images around the clock
- **Intuitive:** Instrument operation and data analysis are easy to perform with no special training required
- **Nonperturbing:** Fluorescent imaging and reagents spanning three channels (red, green, and blue)

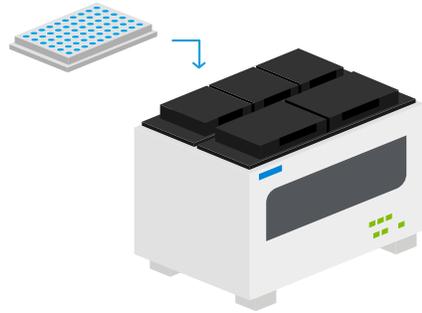
1.

Seed cells into E-Plate VIEW 96
(Adherent or Suspension Cells)



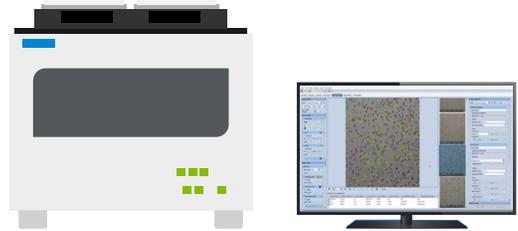
2.

Load E-Plates into xCELLigence
RTCA eSight Instrument



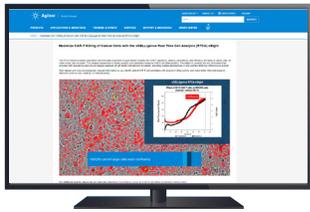
3.

Add treatments according to
experimental requirements

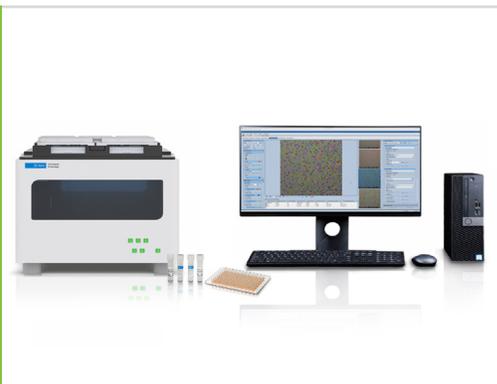


4.

Simultaneous Monitoring:
Real-time impedance monitoring
and live-cell imaging



Drive New Insights and Key Discoveries with New Live Cell Analysis Applications for eSight

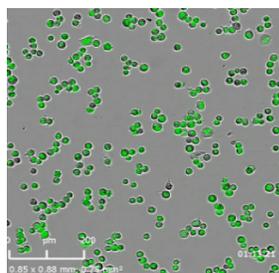


Cell Health and Viability

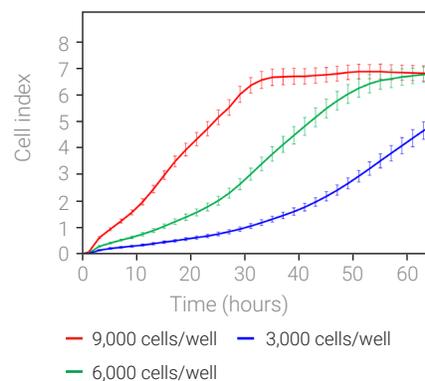
Proliferation

Label nuclei for cell counts and measure growth or growth inhibition. Count living cells in real-time by Agilent eLenti or eLive dyes or via label-free impedance.

Imaging



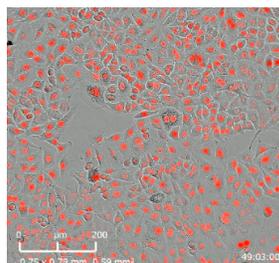
Impedance-based analysis



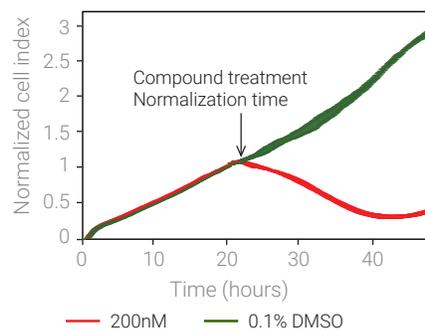
Cytotoxicity

Detect cell death using Agilent eTox reagents. Measure cell viability with simple mix and read protocols, and with multiplexed imaging plus label-free impedance.

Imaging



Impedance-based analysis



Apoptosis

Measure real-time cell health directly and capture early and late apoptotic events such as modulation of cell adhesion, cell membrane integrity, and cell detachment. Utilize Agilent eAnnexin and eCaspase reagents to detect apoptosis, or via label-free impedance.

Imaging

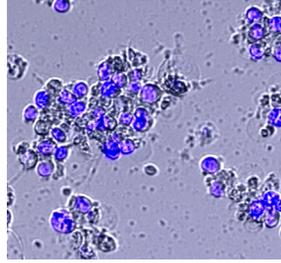
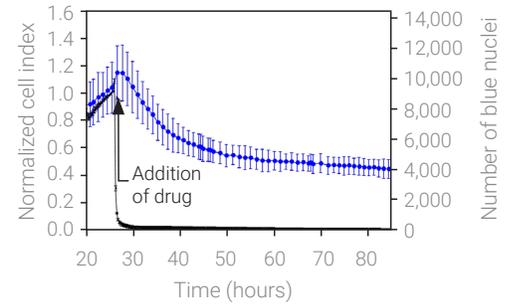


Image and Impedance-based analysis



Cell-by-cell analysis

Continuously monitor and quantify single-cell segmentation by label-free analysis. Gather cell count and nuclear fluorescent recognition count as a function over time. Measure cell subpopulations over time and gain insights into cellular heterogeneity.

Imaging

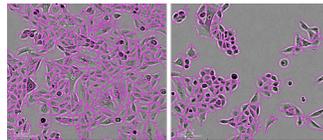
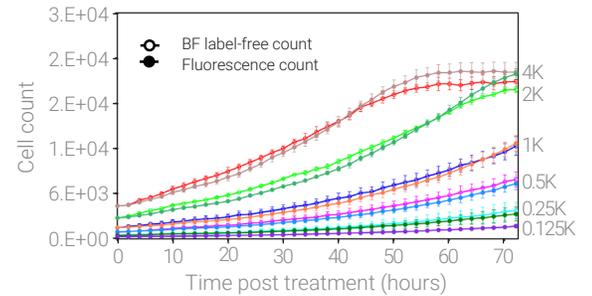


Image-based analysis



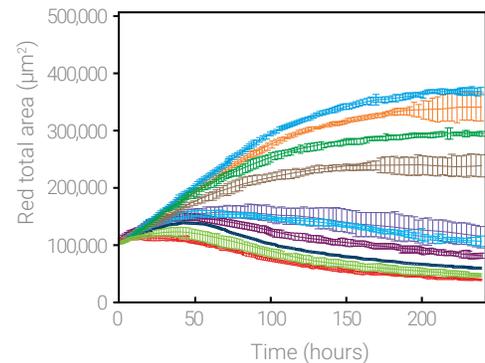
3D tumor spheroids

Continuously monitor and measure 3D tumor spheroid formation, growth, or shrinkage. Gain more insights into various drug or immuno-oncology studies.

Imaging



Image-based analysis



Migration and Invasion

Wound healing migration and invasion

Assess the effects of treatments on cell migration profiles, and investigate the rate of wound closure rate. Explore metastatic potential and how various conditions affect or delay healing. Metrics such as wound width, confluence, cell index, wound count, and density are easily measurable with automated software.

Imaging

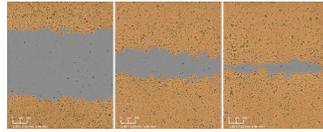
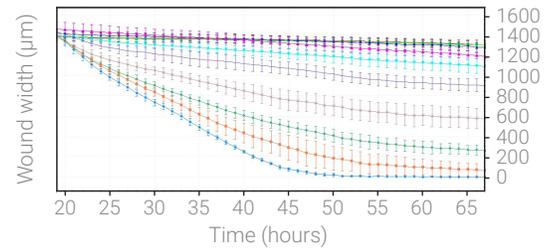


Image and Impedance-based analysis



3D spheroid invasion

Study invasive potential of tumor spheroids over time, as you measure and quantify metastatic potential by label or label-free analysis. Investigate response to treatments and the inhibitory effect on invasion and progression.

Imaging

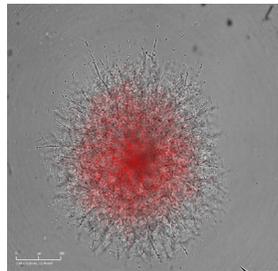
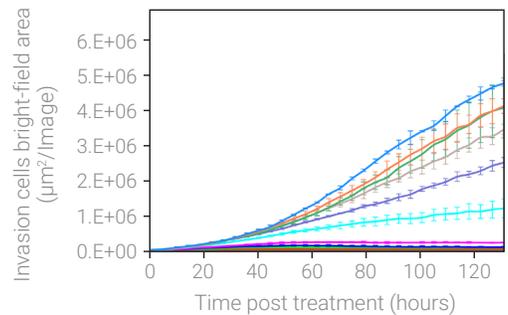


Image-based analysis



Immuno-Oncology and Cell Function

Immunotherapy

Now easily calculate and convert to % cytotoxicity using imaging parameters to expand your Immunotherapy and Immune Cell Killing Analysis and measure potency with ease.

Imaging

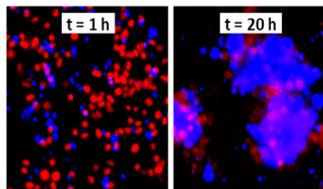
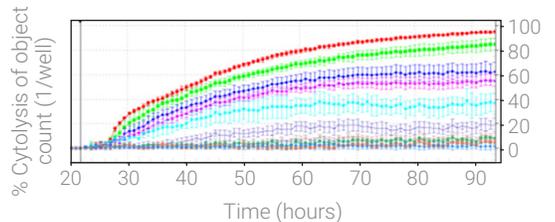


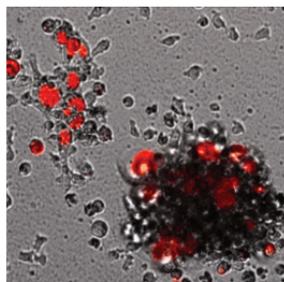
Image-based analysis



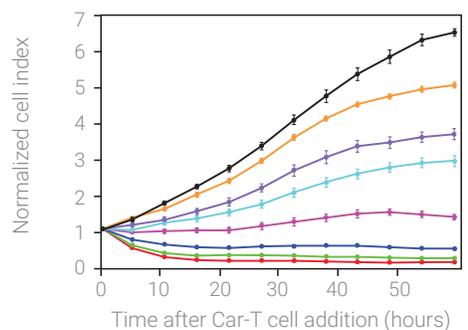
Immune cell killing

Directly measure the potency of immunotherapies against tumor cells with an immune cell killing assay that is simple, quantitative, and high throughput. Get detailed insight into serial killing and exhaustion using NK cells, engineered T cells, antibodies, and more.

Imaging



Impedance-based analysis



Immune cell clustering and activation

Gain more phenotypic insights into what is driving dynamic changes in your immune cells via cell-cell interactions. Easily quantify confluence and object count of immune cell clusters.

Imaging

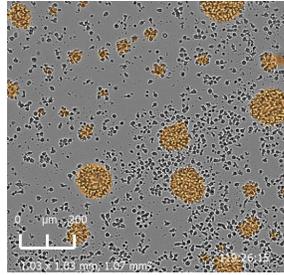
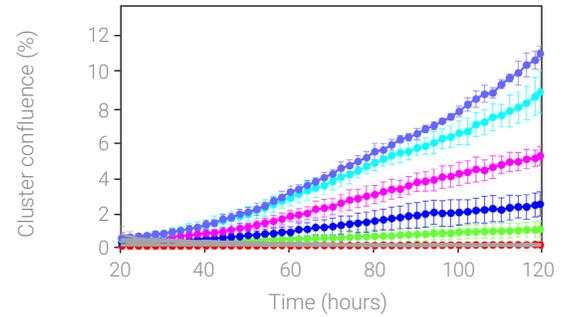


Image-based analysis



Phagocytosis

Dynamically analyze key mechanisms by which the immune system removes pathogens and cell debris. Track the entire process in real-time by imaging, screen for pathway modulators, and discover therapeutic interventions. Label and visualize macrophages via eLenti reagents.

Imaging

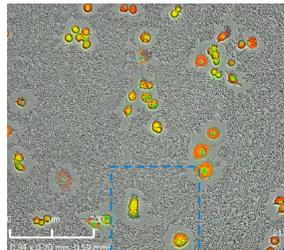
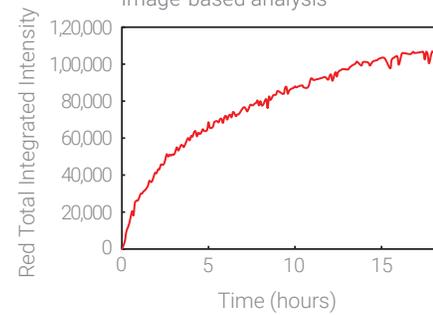


Image-based analysis



Cell Monitoring and other Workflows

Dilution cloning

Easily track cell monoclonal stable cell lines over time using a limited dilution cloning method on eSight. Automatically screen clonal cell lines with minimal effort and high efficiency.

Imaging

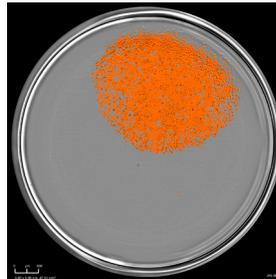
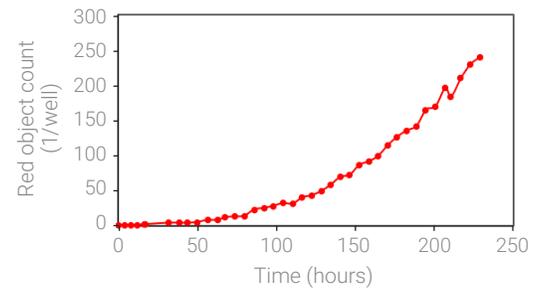
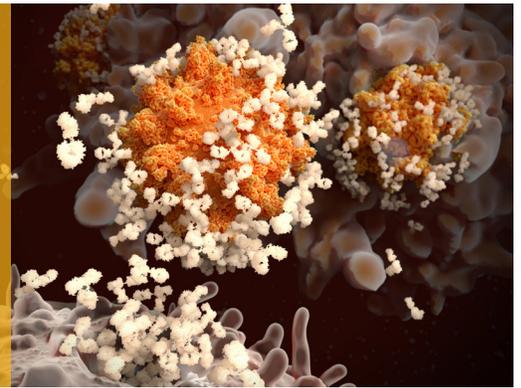


Image-based analysis



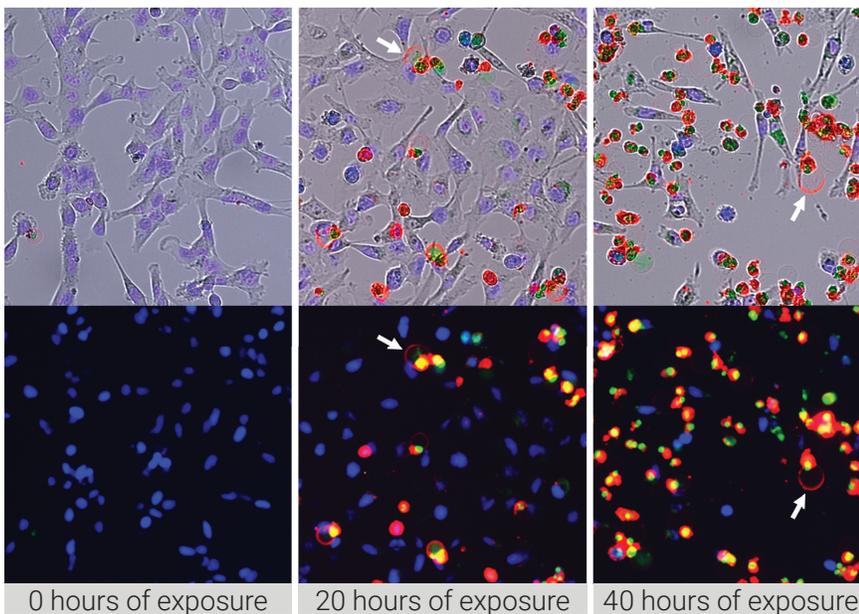
See Cellular Events and Biological Responses in Real-Time



Gain deeper biological insights with Agilent reagents for eSight

The eSight live-cell assays and reagents enable real-time kinetic evaluation of a compound via its concentration and associated time points required to perturb the target. Easily detect and determine the mechanisms of action of cell death and monitor viability with Agilent reagents for the eSight. Results may be validated simultaneously with a label-free approach, giving more confidence in your data.

Real-time impedance and live-cell imaging apoptosis assay



- Red fluorescence: Annexin V
- Green fluorescence: Activated Caspase 3
- Blue fluorescence: Nuclear-localized BFP (Blue fluorescent protein)

With *three distinct fluorescent channels*, and Agilent reagents such as eCaspase-3, eAnnexin V, and eLive, the RTCA esight provides a direct way to assess the extent of tumor cell killing via apoptosis or in addition to other markers of T cell activation/exhaustion.

| Application | Product | Part number |
|---|---------------------------------|-------------|
| Proliferation, Activation, and Cell Counting Visualize and quantify living cells in real-time without removing cultures Learn more | eLive Red | 8711004 |
| | eLive Green | 8711003 |
| | eLive Enhancer (Verapamil) | 8711038 |
| | eLenti Green (EF-1 Alpha, Puro) | 8711010 |
| | eLenti Red (EF-1 Alpha, Puro) | 8711011 |
| | eLenti Blue (EF-1 Alpha, Puro) | 8711012 |
| Cytotoxicity Detect and count non-viable cells with simple mix-and-read protocols Learn more | eTox Green | 8711008 |
| | eTox Red | 8711009 |
| Apoptosis Quantify apoptosis in real-time with simple mix-and-read protocols Learn more | eAnnexin V Blue | 8711026 |
| | eAnnexin V Red | 8711007 |
| | eAnnexin V Green | 8711006 |
| | eCaspase Green | 8711005 |
| | eCaspase Blue | 8711027 |
| Immune Cell Killing and 3D Spheroids Detect tumor cell death directly and continuously analyze in 2D or 3D Learn more | eAnnexin V Blue | 8711026 |
| | eAnnexin V Red | 8711007 |
| | eAnnexin V Green | 8711006 |
| | eCaspase Green | 8711005 |
| | eCaspase Blue | 8711027 |

These reagents can be combined with other fluorescent live-cell imaging reagents for multiplexed measurements including cell viability, apoptosis, and cytotoxicity all in a single well.

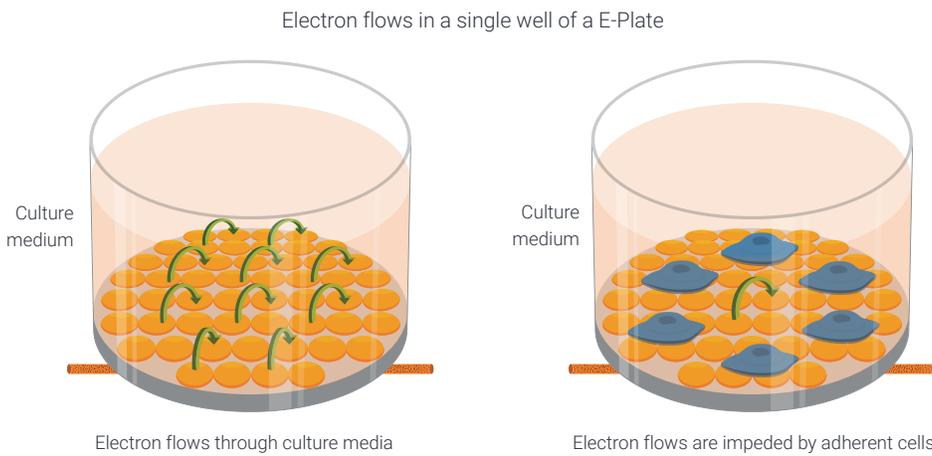
Learn more at: www.agilent.com/chem/esight-reagents

Combine the power of a label-free cellular impedance readout with imaging—boost your confidence and get more insightful data with ease

How does impedance work?

Agilent xCELLigence RTCA instruments use gold biosensors embedded in the bottom of specialized microplate wells (Agilent E-Plates) to noninvasively monitor cell status including cell number, cell size, and cell-substrate attachment quality. This technology enables enhanced sensitivity, the preclusion of labels, and kinetic measurement of cell health and behavior. This proprietary design enables large populations of cells to be monitored simultaneously.

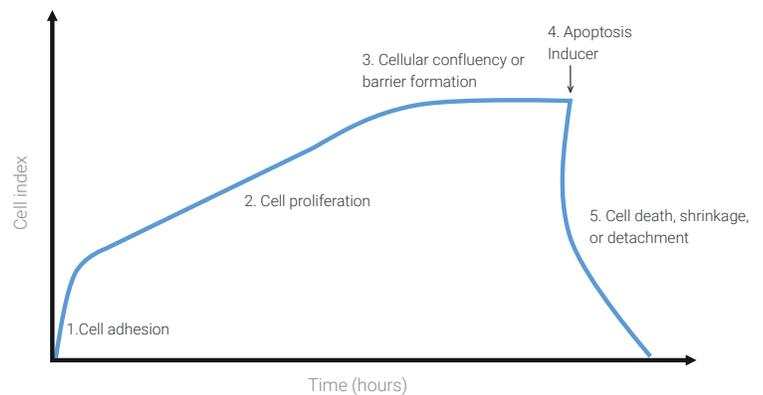
Cellular impedance



The biosensors detect cellular impedance as cells adhere to and proliferate on the E-Plates. The sensors provide an extremely sensitive readout of cell number, size, morphology, and substrate attachment quality in real-time. Capture an entire 96-well plate in just 3 seconds.

When cells have been added to the wells, there is a rapid increase in impedance via cell adhesion and attachment (1). Cells begin to undergo proliferation and increase in number (2). When cells reach confluency, the Cell Index (CI) value plateaus (3).

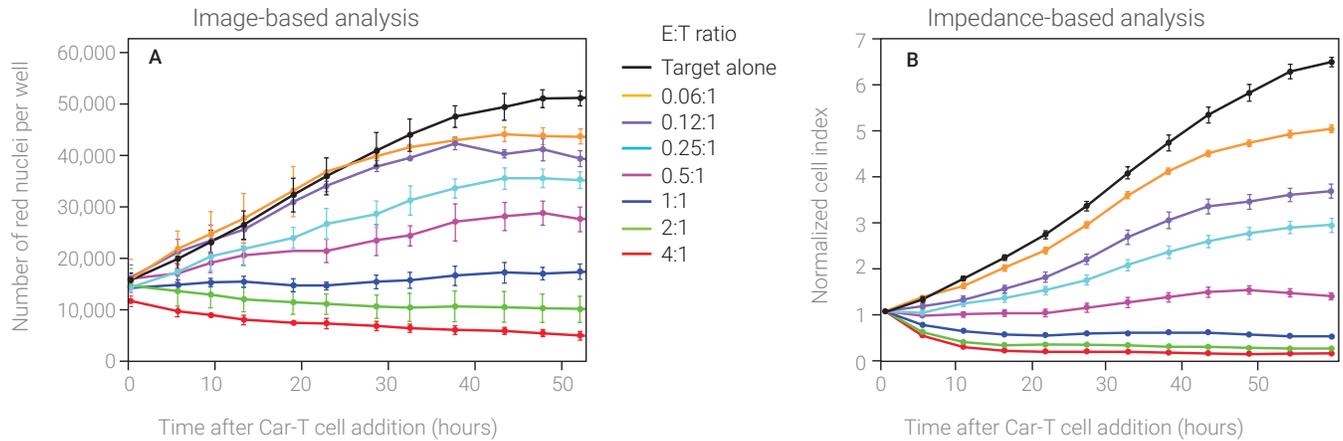
Addition of an apoptotic inducer or drug (4), causes CI to decrease back to zero. Cells begin to round and detach from the well bottom, as they undergo death (5). Impedance-based assays are very flexible and can interrogate a wide variety of phenomena across the full spectrum of cell densities.



Optimize your immune cell killing with multiparametric impedance and imaging readouts—capture what you’re missing

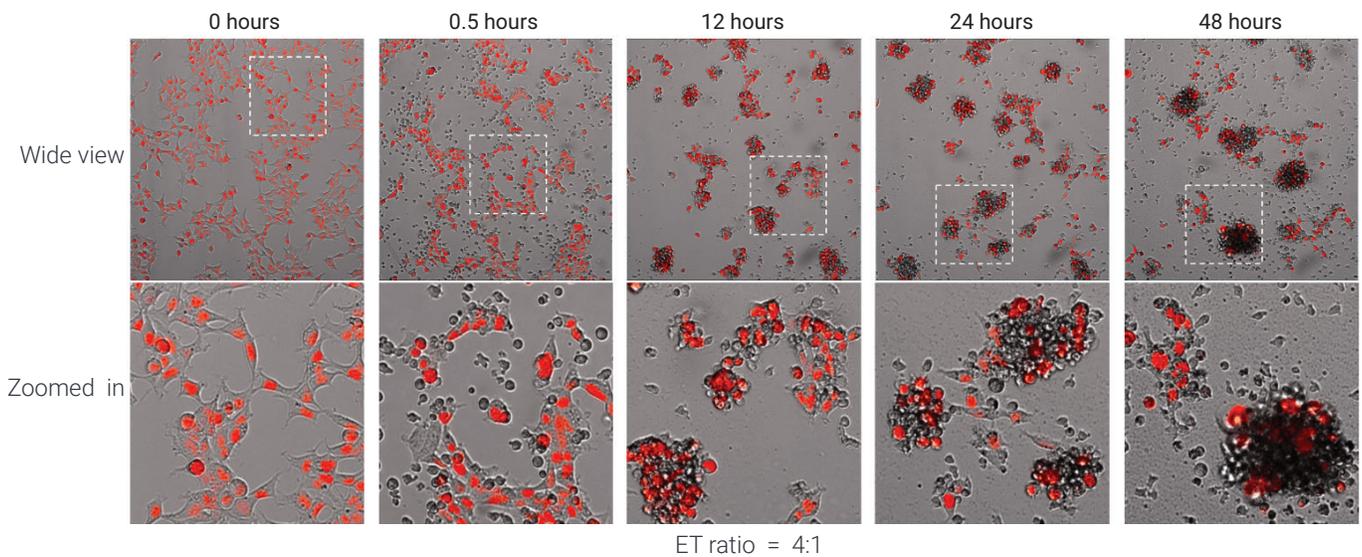
Impedance biosensors provide a simple, label-free assessment of tumor cell viability upon co-culture with effector cells such as CAR-T.

Gain a qualitative and quantitative assessment of cell morphology, confluence, and aggregation under various treatment and immune-cell killing assay conditions.



Time courses for HEK-293-CD19 killing by CD19 CAR-T cells as measured by imaging (A) and impedance (B). Assays were run in triplicate; error bars represent standard deviation.

Profile biological activity over time with multiple imaging channels



Killing of RFP-expressing HEK-293-CD19 cells by CD19 Car-T cells. Comparison of different time points post-Car-T cell addition at a constant E:T ratio of 4:1. The white squares in the upper panels denote the regions that are blown up in the lower panels. The unlabeled Post-CAR-T cells are grey.

Additional eSight application resources

- Proliferation - [Monitoring Cell Proliferation in Real-time Using the Agilent xCELLigence RTCA eSight](#)

- Apoptosis - [Combining Live-Cell Imaging with Cellular Impedance to Monitor Apoptotic Cell Death in Real-Time](#)

- Cytotox - [Real-Time Antibody-Dependent Cell-Mediated Cytotoxicity \(ADCC\) Assays](#)

- Immune Cell Killing - [Real-Time Potency Assay for Car-T Cell Killing of Adherent Cancer Cells](#)
- [Bispecific T Cell Engager \(BiTE\) Mediated Cancer Cell Lysis Assay](#)

- Phagocytosis - [Real-Time Visualization and Quantitative Analysis of Macrophage Phagocytosis Using the xCELLigence RTCA eSight](#)

- Virology - [Detecting and Characterizing Virus Neutralizing Antibodies in Real-Time Using Cellular Impedance and Live-Cell Imaging](#)
- [Screening and Characterizing Antiviral Drugs in Real Time Using xCELLigence RTCA eSight's Combination of Impedance and Imaging](#)

- Cell Stress - [Real-time, Multiday Cell Stress Assay](#)

- 3D Tumor Spheroid - [Monitoring Drug-Mediated 3D Tumor Spheroid Shrinkage Using xCELLigence RTCA eSight](#)

- Immune Cell Fitness and Function - [Metabolic Preconditioning Improves Engineered T Cell Fitness and Function](#)

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