
Vala
SCIENCES
HTS Assays
Kinetic Image Cytometer®

TABLE OF CONTENTS

INTRODUCTION

Vala Sciences helps researchers achieve an unrivaled *in vitro* understanding of how human cells respond to drug compounds.

Vala is committed to providing expert services to pharmaceutical developers and researchers to help overcome the persistent challenge of reducing avoidable clinical failures of drug candidates.

Breakthrough Single-Cell Video Analysis Platform

By measuring up to three channels (voltage, Ca+ and contractility) of single-cell hiPSC kinetic data in real time and comparing cell response with known healthy or disease-model cells, Vala can help rank order drug candidates by identifying toxicities, dose response and efficacy.

Platform Enabled CRO Services

Vala delivers expert services that leverage our flagship Kinetic Image Cytometer (KIC®), CyteSeer® Cellular Analysis Algorithms and ValaDATE® AI Platform (in development).

Company History

Vala Sciences was founded in 2004 by Jeffrey Price, M.D., Ph.D. in San Diego. Dr. Price, a pioneer in high throughout cell imaging and data analysis, previously founded and led Q3DM until its acquisition by Beckman Coulter Inc. in 2003. Since its inception, Vala has worked closely with academic leaders, government health institutions and top pharmaceutical scientists to advance the state of cell imaging, analysis and interpretation for drug candidate screening.

Vala Sciences is privately held.

KIC® Assays

Vala Sciences offers the following Kinetic Image Cytometry® and High Content Analysis assays to test for drug efficacy and toxicity. Contact us to learn more about our screening services or to request a custom assay.

CARDIO ASSAYS

1. CARDIOTOXIC EFFECTS ON CALCIUM TRANSIENTS IN HIPSC-DERIVED CARDIOMYOCYTES

Vala Assay: #CT1

Cell Type: Human cardiomyocytes derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on calcium transient kinetics in human cardiomyocytes derived from induced pluripotent stem cells. Transient increases in cardiomyocyte intracellular calcium concentration translate the cardiac action potential into contractile force. Disruptions in calcium transients can disrupt contractile activity. This assay measures the fluorescence of calcium indicator Fluo-4 at 30 Hz for 10 seconds to capture calcium transient activity on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of calcium transient kinetic parameters (transient duration at multiple decay points, rise and decay times, upstroke and downstroke velocities, etc.) to provide a comprehensive picture of how each compound affects the cardiac calcium transient.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number)
- Fluorescence channel #2 (green channel): Fluo-4 (calcium transient kinetic measurements)

2. CARDIOTOXIC EFFECTS ON ACTION POTENTIALS IN HIPSC-DERIVED CARDIOMYOCYTES

Vala Assay: #CT2

Cell Type: Human cardiomyocytes derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on action potential kinetics in human cardiomyocytes derived from induced pluripotent stem cells. Transient electrical depolarizations, or action potentials, direct cardiomyocyte contractile activity, and disruptions in action potentials can disrupt contraction. This assay measures the fluorescence of membrane potential indicator FluoVolt at 30 Hz for 10 seconds to capture action potentials on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of action potential kinetic parameters (duration at multiple decay points, rise and decay times, upstroke and downstroke velocities, etc.) to provide a comprehensive picture of how each compound affects the cardiac action potential.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number)
- Fluorescence channel #2 (green channel): FluoVolt (action potential kinetic measurements)

3. CARDIOTOXIC EFFECTS ON CALCIUM TRANSIENTS AND CONTRACTILITY IN HIPSC-DERIVED CARDIAC MYOCYTES

Vala Assay: #CT3

Cell Type: Human cardiomyocytes derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on calcium transient kinetics and contractility in human cardiomyocytes derived from induced pluripotent stem cells. Transient increases in cardiomyocyte intracellular calcium concentration translate the cardiac action potential into contractile force. Disruptions in calcium transients can disrupt contractile activity. This assay measures the fluorescence of calcium indicator Fluo-4 at 30 Hz for 10 seconds to capture calcium transient activity on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of calcium transient kinetic parameters (transient duration at multiple decay points, rise and decay times, upstroke and downstroke velocities, etc.) to provide a comprehensive picture of how each compound affects the cardiac calcium transient. CyteSeer® also detects the motion of Fluo-4-labeled features within each cardiomyocyte to report kinetic measurements on the contraction that results from each calcium transient.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number)
- Fluorescence channel #2 (green channel): Fluo-4 (calcium transient and contractile activity kinetic measurements)

4. CARDIOTOXIC EFFECTS ON ACTION POTENTIALS AND CONTRACTILITY IN HIPSC-DERIVED CARDIAC MYOCYTES

Vala Assay: #CT4

Cell Type: Human cardiomyocytes derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on action potential kinetics and contractility in human cardiomyocytes derived from induced pluripotent stem cells. Transient electrical depolarizations, or action potentials, direct cardiomyocyte contractile activity, and disruptions in action potentials can disrupt contraction. This assay measures the fluorescence of membrane potential indicator FluoVolt at 30 Hz for 10 seconds to capture action potentials on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of action potential kinetic parameters (duration at multiple decay points, rise and decay times, upstroke and downstroke velocities, etc.) to provide a comprehensive picture of how each compound affects the cardiac action potential. CyteSeer® also detects the motion of FluoVolt-labeled features within each cardiomyocyte to report kinetic measurements on the contraction that results from each action potential.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number)
- Fluorescence channel #2 (green channel): FluoVolt (action potential and contractile activity kinetic measurements)

5. CARDIOTOXIC EFFECTS ON CALCIUM TRANSIENTS AND CONTRACTILITY IN ADULT CANINE CARDIOMYOCYTES

Vala Assay: #CT5

Cell Type: Cardiomyocytes isolated from adult canines

Main Goal of Assay: Determine the effects of test compounds on calcium transient kinetics and contractility in cardiomyocytes from adult dogs, which have similar heart rates and cardiac electrophysiology to humans. Transient increases in cardiomyocyte intracellular calcium concentration translate the cardiac action potential into contractile force. Disruptions in calcium transients can disrupt contractile activity. This assay measures the fluorescence of calcium indicator Fluo-4 at 30 Hz for 10 seconds to capture calcium transient activity on a cell-by-cell basis for electrically paced cardiomyocytes. Vala's CyteSeer® image analysis program then reports a range of calcium transient kinetic parameters (transient duration at multiple decay points, rise and decay times, upstroke and downstroke velocities, etc.) to provide a comprehensive picture of how each compound affects the cardiac calcium transient. CyteSeer® also detects changes in cardiomyocyte length to report kinetic measurements on the contraction that results from each calcium transient.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number)
- Fluorescence channel #2 (green channel): Fluo-4 (calcium transient and contractile activity kinetic measurements)

6. CARDIOTOXIC EFFECTS ON ACTION POTENTIALS AND CONTRACTILITY IN ADULT CANINE CARDIOMYOCYTES

Vala Assay: #CT6

Cell Type: Cardiomyocytes isolated from adult canines

Main Goal of Assay: Determine the effects of test compounds on action potential kinetics and contractility in cardiomyocytes from adult dogs, which have similar heart rates and cardiac electrophysiology to humans. Transient electrical depolarizations, or action potentials, direct cardiomyocyte contractile activity, and disruptions in action potentials can disrupt contraction. This assay measures the fluorescence of membrane potential indicator FluoVolt at 30 Hz for 10 seconds to capture action potentials on a cell-by-cell basis for electrically paced cardiomyocytes. Vala's CyteSeer® image analysis program then reports a range of action potential kinetic parameters (duration at multiple decay points, rise and decay times, upstroke and downstroke velocities, etc.) to provide a comprehensive picture of how each compound affects the cardiac action potential. CyteSeer® also detects changes in cardiomyocyte length to report kinetic measurements on the contraction that results from each action potential.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number)
- Fluorescence channel #2 (green channel): FluoVolt (action potential kinetic measurements)

7. CARDIOTOXIC EFFECTS ON SARCOMERE CONTRACTION IN LABEL-FREE ADULT CANINE CARDIOMYOCYTES

Vala Assay: #CT7

Cell Type: Cardiomyocytes isolated from adult canines

Main Goal of Assay: Determine the effects of test compounds on sarcomere contraction in cardiomyocytes from adult dogs, which have similar heart rates and cardiac electrophysiology to humans. In cardiomyocytes, contractile proteins are organized into repeating structures called sarcomeres that coordinate force production across the cell. This assay detects changes in sarcomere spacing in brightfield images captured at 30 Hz for 10 seconds on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of sarcomere shortening kinetic parameters (duration at multiple decay points, rise and decay times, upstroke and downstroke velocities, etc.) to provide a comprehensive picture of how each compound affects intracellular cardiomyocyte contraction.

CyteSeer® Data Readout

- Brightfield: changes in sarcomere spacing over time

NEURO ASSAYS

1. CALCIUM TRANSIENTS IN HIPSC-GLUTAMATERGIC NEURONS, MONOCULTURE

Vala Assay: #NT1

Cell Type: Human glutamatergic neurons derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on calcium transient activity in human glutamatergic neurons derived from induced pluripotent stem cells. Neurons exhibit action potential-dependent and independent peaks in intracellular calcium concentration that regulate neuronal health and function. Dysregulation of neuronal calcium concentration occurs in aging, traumatic brain injury, and neurodegenerative diseases.

This assay measures the fluorescence of calcium indicator Rhod-4 at 4 Hz for 2 minutes to capture neuronal calcium transient activity on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of parameters (percent of active cells, event frequency, mean and maximum peak amplitudes, peak width, etc.) to provide a comprehensive picture of how each compound affects neuronal calcium activity. This assay can be run concurrently with the microglia co-culture assay #NT2 to determine if the presence of microglia alters the neuronal calcium response to compound treatment.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (red channel): Rhod-4 (neuronal calcium transient parameters)

2. CALCIUM TRANSIENTS IN HIPSC- GLUTAMATERGIC NEURONS, MICROGLIA CO-CULTURE

Vala Assay: #NT2

Cell Types: Human glutamatergic neurons derived from hiPSCs in co-culture with human microglia derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on calcium transient activity in co-cultures of human glutamatergic neurons and human microglia, both derived from induced pluripotent stem cells. Neurons exhibit action potential-dependent and -independent peaks in intracellular calcium concentration that are dysregulated in aging, traumatic brain injury, and neurodegenerative diseases. Microglia, the main central nervous system immune cells, also exhibit transient increases in intracellular calcium concentration, especially under pathological conditions.This assay measures the fluorescence of calcium indicator Rhod-4 at 4 Hz for 2 minutes to capture neuronal and microglial calcium transient activity on a cell-by-cell basis. After live imaging, the cells are fixed, immunolabeled, and imaged for cell-specific markers (e.g., βIII tubulin for neurons and IBA1 for microglia). Vala's CyteSeer® image analysis program first aligns the live and immunofluorescence images to confirm the identity of each cell. CyteSeer® then reports a range of parameters (percent of active cells, event frequency, mean and maximum peak amplitudes, peak width, etc.) to provide a comprehensive picture of how each compound affects neuronal and microglial calcium activity.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (green channel, βIII-tubulin): neuron number and morphology
- Fluorescence channel #3 (red channel): Rhod-4 (calcium transient parameters)
- Fluorescence channel #4 (far red channel, IBA1): microglia number and morphology

3. CALCIUM TRANSIENTS IN HIPSC-DOPAMINERGIC MODEL OF PARKINSON'S DISEASE

Vala Assay: #NT3

Cell Type: Human dopaminergic neurons derived from normal hiPSCs or hiPSCs with the A53T alpha-synuclein mutation introduced with genome editing

Main Goal of Assay: Determine the effects of test compounds on calcium transient activity in human dopaminergic neurons derived from induced pluripotent stem cells. Neurons exhibit action potential-dependent and independent peaks in intracellular calcium concentration that regulate neuronal health and function. Dysregulation of neuronal calcium concentration occurs in aging, traumatic brain injury, and neurodegenerative diseases.

This assay features dopaminergic neurons, which are affected in Parkinson's Disease, derived from normal hiPSCs or from isogenic hiPSCs with the A53T alpha-synuclein mutation introduced with genome editing. The A53T alpha-synuclein mutation causes early-onset Parkinson's Disease. Testing compound effects on calcium transients in both cell types can provide insight into Parkinson's disease mechanisms and identify potential Parkinson's therapeutics.

This assay measures the fluorescence of calcium indicator Rhod-4 at 4 Hz for 2 minutes to capture neuronal calcium transient activity on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of parameters (percent of active cells, event frequency, mean and maximum peak amplitudes, peak width, etc.) to provide a comprehensive picture of how each compound affects neuronal calcium activity. This assay can be run concurrently with the neuronal action potential assay #NT4 to compare compound effects on calcium and voltage activity.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (red channel): Rhod-4 (neuronal calcium transient parameters)

4. ACTION POTENTIALS IN HIPSC-DOPAMINERGIC MODEL OF PARKINSON'S DISEASE

Vala Assay: #NT4

Cell Type: Human dopaminergic neurons derived from normal hiPSCs or hiPSCs with the A53T alpha-synuclein mutation introduced with genome editing

Main Goal of Assay: Determine the effects of test compounds on action potential activity in human dopaminergic neurons derived from induced pluripotent stem cells. Neurons communicate with target cells through transient membrane electrical depolarization called action potentials. Dysregulation of action potential frequency can impede neuronal communication and cause the calcium overload, oxidative stress, inflammation, and cell death behind neurodegenerative diseases.

This assay features dopaminergic neurons, which are affected in Parkinson's Disease, derived from normal hiPSCs or from isogenic hiPSCs with the A53T alpha-synuclein mutation introduced with genome editing. The A53T alpha-synuclein mutation causes early-onset Parkinson's Disease. Testing compound effects on action potentials in both cell types can provide insight into Parkinson's disease mechanisms and identify potential Parkinson's therapeutics.

This assay measures the fluorescence of voltage indicator FluoVolt at 60 Hz for 10 seconds to capture neuronal action potentials on a cell-by-cell basis. Vala's CyteSeer® image analysis program then reports a range of parameters (percent of active cells, event frequency, mean and maximum peak amplitudes, peak width, etc.) to provide a comprehensive picture of how each compound affects neuronal action potentials. This assay can be run concurrently with the neuronal calcium transient assay #NT3 to compare compound effects on calcium and voltage activity.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (green channel): FluoVolt (neuronal action potential parameters)

5. NEURITE MORPHOLOGY AND SYNAPSE DENSITY IN HIPSC- GLUTAMATERGIC NEURONS, MONOCULTURE

Vala Assay: #NT5

Cell Type: Human glutamatergic neurons derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on neurite morphology and synapse density in human glutamatergic neurons derived from induced pluripotent stem cells. Neurons communicate by forming dense networks of long, thin neurites that contain synapses, specialized structures that transmit chemical and/or electrical signals between neurons. Aging, neurodegenerative diseases, and other central nervous system pathologies can change neurite morphology and/or synapse density, thus interfering with communication across neuronal networks.

This fixed-endpoint assay uses images of neurons immunolabeled for neuron-specific βIII tubulin, the presynaptic marker SV2, and the postsynaptic marker PSD95. Vala's CyteSeer® image analysis program first traces βIII tubulin-positive neurites and calculates their length. CyteSeer® then identifies synapses as SV2-positive presynapses near both PSD95-positive postsynapses and βIII tubulin-positive neurites. This assay can be run concurrently with Vala Assay #NT6 microglia co-culture assay to determine if the presence of microglia alter the neuronal response to compound treatment.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (green channel, SV2): presynapse location, count, size, and SV2 intensity
- Fluorescence channel #3 (red channel, βIII tubulin): neurite length and morphology
- Fluorescence channel #4 (far red channel, PSD95): postsynapse location, count, size, and PSD95 intensity

6. NEURITE MORPHOLOGY IN HIPSC-GLUTAMATERGIC NEURONS, MICROGLIA CO-CULTURE

Vala Assay: #NT6

Cell Type: Human glutamatergic neurons derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on neurite morphology in human glutamatergic neurons in co-culture with human microglia, both derived from induced pluripotent stem cells. Neurons communicate by forming dense networks of long, thin neurites. Aging, neurodegenerative diseases, and other central nervous system pathologies can change neurite morphology, thus interfering with communication across neuronal networks. Microglia, the main central nervous system immune cells, may regulate neurite length and morphology in health and disease.

This fixed-endpoint assay uses images of neuron/microglia co-cultures immunolabeled for neuron-specific βIII tubulin and microglia-specific IBA1. Vala's CyteSeer® image analysis program traces βIII tubulin-positive neurites and calculates their length and then identifies IBA1-positive microglia and reports a range of morphology parameters.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (green channel, βIII tubulin): neuron number, neurite length and morphology
- Fluorescence channel #3 (red channel, IBA1): microglia number and morphology

7. PHAGOCYTOSIS BY HIPSC-MICROGLIA, MONOCULTURE

Vala Assay: #NT7

Cell Type: Human microglia derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on the rate that microglia phagocytose or engulf particles such as zymosan-coated beads or β-amyloid oligomers. Microglia, the main central nervous system immune cells, can exert neuroprotective effects by clearing pathogens and cellular debris, pruning synapses, and eliminating protein aggregates. Neuroinflammation and neurodegenerative diseases can increase microglia engulfment activity.

This assay measures the intensity of fluorescently labeled zymosan beads or β-amyloid oligomers within microglia every 5 minutes for 12 hours to capture microglial particle phagocytosis or engulfment on a cell-by-cell basis over time. Vala's CyteSeer® image analysis program reports the total intensity of engulfed particles and the rate of engulfment over time. This assay can be run concurrently with Vala Assay #NT8 neuron co-culture assay to determine if the presence of neurons alter the microglial response to compound treatment.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (green channel, CellTracker): microglia boundaries and morphology
- Fluorescence channel #3 (red channel, fluorescently labeled beads or particles): intensity of phagocytosed or engulfed particle

8. PHAGOCYTOSIS BY HIPSC-MICROGLIA, NEURON CO-CULTURE

Vala Assay: #NT8

Cell Type: Human microglia derived from hiPSCs in co-culture with glutamatergic neurons derived from hiPSCs

Main Goal of Assay: Determine the effects of test compounds on the rate that microglia phagocytose or engulf particles such as zymosan-coated beads or β-amyloid oligomers. Microglia, the main central nervous system immune cells, can exert neuroprotective effects by clearing pathogens and cellular debris, pruning synapses, and eliminating protein aggregates. Neuroinflammation and neurodegenerative diseases can increase microglia engulfment activity.

This assay measures the intensity of fluorescently labeled zymosan beads or β-amyloid oligomers within microglia every 5 minutes for 12 hours to capture microglial particle engulfment on a cell-by-cell basis over time. Vala's CyteSeer® image analysis program reports the total intensity of engulfed particles and the rate of engulfment over time. This assay is run on microglia in co-culture with glutamatergic neurons derived from hiPSCs.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): Hoechst (cell number, viability)
- Fluorescence channel #2 (green channel, CellTracker): microglia boundaries and morphology
- Fluorescence channel #3 (red channel, fluorescently labeled beads or particles): intensity of phagocytosed or engulfed particle

ADIPOGENESIS-LIPOLYSIS ASSAYS

1. ADIPOGENESIS

Vala Assay: #A1

Cell Type: Primary human subcutaneous adipocytes

Main Goal of Assay: Determine test compound effects on adipogenesis in primary human preadipocytes. Fat accumulation is highly correlated with heart disease, fatty liver disease, and atherosclerosis. Reduced adipogenesis contributes to cachexia (wasting disease) associated with cancer and HIV infection. When cultured in media that increases cyclic AMP and glucocorticoid hormones, preadipocytes differentiate into mature adipocytes, which feature large lipid droplets and express adipocyte biomarkers (e.g., ADFP and perilipin). Determining compound effects on adipogenesis can reveal their role in overall health, metabolism, and organ function.

CyteSeer® Data Readout

- Bright field: rate of lipid droplet growth over 14 to 21 days
- Fluorescence channel #1 (DAPI): cell number, ploidy
- Fluorescence channel #2 (green channel, lipid): Lipid droplet count, area, subcellular distribution
- Fluorescence channel #3 (red channel, perilipin): Expression level and colocalization with the lipid droplets of perilipin, a protein that participates in fat metabolism
- Fluorescence channel #4 (far red channel, ADFP): Expression level and colocalization with the lipid droplets of ADFP, a protein thought to initiate lipid droplet formation

2. ADIPOGENESIS ANTAGONISTS

Vala Assay: #A2

Cell Type: Primary human subcutaneous adipocytes

Main Goal of Assay: Determine test compound effects on adipogenesis in primary human preadipocytes. Fat accumulation is highly correlated with heart disease, fatty liver disease, and atherosclerosis. Reduced adipogenesis contributes to cachexia (wasting disease) associated with cancer and HIV infection. When cultured in media that increases cyclic AMP and glucocorticoid hormones, preadipocytes differentiate into mature adipocytes, which feature large lipid droplets and express adipocyte biomarkers (e.g., ADFP and perilipin). This version of the adipogenesis assay is performed in the presence of 100 nM rosiglitazone (or a PPAR-γ agonist with similar effects) and is designed to specifically detect adipogenesis inhibitors.

CyteSeer® Data Readout

- Bright field: rate of lipid droplet growth
- Fluorescence channel #1 (DAPI): cell number, ploidy
- Fluorescence channel #2 (green channel, lipid): Lipid droplet count, area, subcellular distribution
- Fluorescence channel #3 (red channel, perilipin): Expression level and colocalization with the lipid droplets of perilipin, a protein that participates in fat metabolism
- Fluorescence channel #4 (far red channel, ADFP): Expression level and colocalization with the lipid droplets of ADFP, a protein thought to initiate lipid droplet formation

3. LIPOLYSIS

Vala Assay: #L1

Cell Type: Primary human subcutaneous adipocytes

Main Goal of Assay: Determine test compound effects on hormone sensitive lipase (HSL) translocation and perilipin phosphorylation in mature human adipocytes. When metabolic energy needs increase, lipid droplets within adipocytes are subject to lipolysis, in which triglycerides are broken down into fatty acids and glycerol. Lipolysis involves the phosphorylation of perilipin, a protein that coats lipid droplets, and the phosphorylation and translocation of HSL to the droplets. Determining compound effects on lipolysis can reveal their role in overall health, metabolism, and organ function.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): toxicity (cell loss and altered nuclear morphology)
- Fluorescence channel #2 (green channel): lipid droplets (number, size, and morphology)
- Fluorescence channel #3 (red channel): phospho-perilipin (expression level and colocalization with lipid droplets)
- Fluorescence channel #4 (far red channel): phospho-HSL (expression level and colocalization with lipid droplets)

4. LIPOLYSIS ANTAGONISTS

Vala Assay: #L2

Cell Type: Primary human subcutaneous adipocytes

Main Goal of Assay: Determine test compound effects on hormone sensitive lipase (HSL) translocation and perilipin phosphorylation in mature human adipocytes. When metabolic energy needs increase, lipid droplets within adipocytes are subject to lipolysis, in which triglycerides are broken down into fatty acids and glycerol. Lipolysis involves the phosphorylation of perilipin, a protein that coats lipid droplets, and the phosphorylation and translocation of HSL to the droplets. This version of the lipolysis assay is performed in the presence of isoproterenol, which strongly activates HSL and perilipin phosphorylation. Compounds are tested for their ability to inhibit or enhance the isoproterenol response.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): toxicity (cell loss and altered nuclear morphology)
- Fluorescence channel #2 (green channel): lipid droplets (number, size, and morphology)
- Fluorescence channel #3 (red channel): phospho-perilipin (expression level and colocalization with lipid droplets)
- Fluorescence channel #4 (far red channel): phospho-HSL (expression level and colocalization with lipid droplets)

5. LIPOLYSIS, MURINE 3T3L1 CELLS

Vala Assay: #L3

Cell Type: Murine 3T3L1 cell-derived adipocytes

Main Goal of Assay: Determine test compound effects on hormone sensitive lipase (HSL) translocation and perilipin phosphorylation in murine 3T3L1 adipocytes. When metabolic energy needs increase, lipid droplets within adipocytes are subject to lipolysis, in which triglycerides are broken down into fatty acids and glycerol. Lipolysis involves the phosphorylation of perilipin, a protein that coats lipid droplets, and the phosphorylation and translocation of HSL to the droplets. Because murine and human adipocytes differ in their lipolytic responses to certain agents, this version of the assay reports compound effects specific to murine metabolism.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): toxicity (cell loss and altered nuclear morphology)
- Fluorescence channel #2 (green channel): lipid droplets (number, size, and morphology)
- Fluorescence channel #3 (red channel): phospho-perilipin (expression level and colocalization with lipid droplets)
- Fluorescence channel #4 (far red channel): phospho-HSL (expression level and colocalization with lipid droplets)

6. LIPID DROPLET FORMATION, HUH-7 CELLS

Vala Assay: #LD2

Cell Type: Human HuH-7 cells

Main Goal of Assay: Determine test compound effects on endogenous lipid droplet content in HuH-7 cells. Fatty liver disease is associated with increased lipid droplet formation within hepatocytes. HuH-7 cells are a human hepatocellular carcinoma-derived cell line with a moderate number of endogenous lipid droplets. Compounds that affect triglyceride formation or induce metabolic stress can affect lipid droplet number, size, and/or morphology. This assay also quantifies expression of ADFP, the major lipid droplet-associated protein in hepatocytes.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): cell number, apoptosis, ploidy
- Fluorescence channel #2 (green channel): lipid droplets (number, size, and morphology)
- Fluorescence channel #3 (red channel): ADFP (expression level and colocalization with lipid droplets)

MEMBRANE PROTEIN ASSAYS

1. BETA-CATENEIN EXPRESSION

Vala Assay: #BC1

Cell Type: HeLa

Main Goal of Assay: Quantify β-catenin expression and cellular localization, particularly in the membrane, nuclear, and cytoplasmic compartments. Under normal conditions, glycogen synthase kinase 3B (GSK3B) phosphorylates β-catenin, leading to its degradation via the ubiquitination/protease system. Wnt pathway activation inhibits GSK3B, reducing β-catenin phosphorylation and degradation. This leads to increased β-catenin expression and β-catenin translocation to the nucleus, where it increases the transcription of proteins that upregulate mitotic activity. Nuclear β-catenin is associated with increased mitosis and can promote tumor formation and growth.

CyteSeer® Data Readout

- Fluorescence channel #1 DAPI: cell number, ploidy
- Fluorescence channel #2 (green channel): β-catenin
- Fluorescence channel #3 (red channel): N-cadherin, E-cadherin, or pan-cadherin

2. N-CADHERIN EXPRESSION

Vala Assay: #NCAD1

Cell Type: N-cadherin expression

Main Goal of Assay: Quantify test compound effects on N-cadherin expression and cellular localization. Cadherins are cell adhesion molecules that provide a mechanical linkage between cells and support cell differentiation, embryogenesis, and migration. Tumor progression is often associated with an upregulation of N-cadherin, which facilitates tumor cell migration and metastasis. This assay can be multiplexed with pan-cadherin immunolabeling to measure changes in N-cadherin expression relative to other cadherins. This assay can also be multiplexed with the β-catenin assay to provide concurrent information on compound effects on β-catenin expression and distribution.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): cell number, ploidy
- Fluorescence channel #2 (green channel): N-Cadherin
- Fluorescence channel #3 (red channel): pan-cadherin or β-catenin

3. E-CADHERIN EXPRESSION

Vala Assay: #ECAD1

Cell Type: Human A431 cells, derived from epithelial carcinoma

Main Goal of Assay: Quantify test compound effects on E-cadherin expression and cellular localization. Cadherins are cell adhesion molecules that provide a mechanical linkage between cells and support cell differentiation, embryogenesis, and migration. E-cadherin levels regulate cell division, and loss of E-cadherin leads to release of β-catenin, Wnt signaling activation, transcription of proto-oncogenes, and tumorigenesis. This assay can be multiplexed with pan-cadherin immunolabeling to measure changes in E-cadherin expression relative to other cadherins. This assay can also be multiplexed with the β-catenin assay to provide concurrent information on compound effects on β-catenin expression and distribution.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): cell number, ploidy
- Fluorescence channel #2 (green channel): E-Cadherin
- Fluorescence channel #3 (red channel): pan-cadherin or β-catenin

4. VE-CADHERIN EXPRESSION

Vala Assay: #VCAD1

Cell Type: Primary human microvascular endothelial cells

Main Goal of Assay: Quantify test compound effects on VE-cadherin expression and cellular localization. VE-cadherin, the major adherens junction protein in vascular endothelial cells, regulates cell-cell adhesion, angiogenesis, and vascular permeability. VE-cadherin contributes to tumor-induced angiogenesis and transendothelial migration of metastatic cancer cells. This assay can be multiplexed with pan-cadherin immunolabeling to measure changes in VE-cadherin expression relative to other cadherins. This assay can also be multiplexed with the β-catenin assay to provide concurrent information on compound effects on β-catenin expression and distribution.

CyteSeer® Data Readout

- Fluorescence channel #1 (DAPI): cell number, ploidy
- Fluorescence channel #2 (green channel): VE-Cadherin
- Fluorescence channel #3 (red channel): pan-cadherin or β-catenin

5. ZO-1 EXPRESSION

Vala Assay: #ZOOTJP1

Cell Type: Canine MDCK cells, derived from kidney epithelial tissue

Main Goal of Assay: Determine test compound effects on ZO-1 expression and localization. Zonula occuldens protein 1 (ZO-1) is a key component of tight junctions, which control the structure, function, and permeability of epithelial and endothelial cell sheets. ZO-1 also negatively regulates epithelial cell proliferation and the epithelial-mesenchymal transition (EMT). This assay therefore reports compound effects on tight junction and epi/endothelial integrity. This assay can detect either upregulation or downregulation of ZO-1 in MDCK cells.

CyteSeer® Data Readout

- Fluorescence channel #1 DAPI: cell number, ploidy
- Fluorescence channel #2 (green channel): ZO-1

MITOCHONDRIAL FUNCTION ASSAYS

1. MITOCHONDRIAL MEMBRANE POTENTIAL, ACUTE

Vala Assay: #MP1

Cell Type: Human HuH-7 cells, derived from hepatocellular carcinoma

Main Goal of Assay: Assess mitochondrial and cellular health by evaluating mitochondrial membrane potential after 30, 60, and 90 minutes of exposure to test compounds. During apoptosis, mitochondria lose their membrane potential due to collapse of the H+ gradient across the inner mitochondrial membrane. Tetramethyl rhodamine methyl ester (TMRM) is a cell permeant cationic dye that accumulates in the matrix of healthy mitochondria due to the negative membrane potential. When the membrane potential is lost, TMRM uptake into mitochondria is prevented and the fluorescent signal is lost.

CyteSeer® Data Readout

- Fluorescence channel #1 (Hoescht): cell number, nuclear size, ploidy, apoptotic fragmentation
- Fluorescence channel #2 (red channel): TMRM (mitochondrial membrane potential)

2. MITOCHONDRIAL MEMBRANE POTENTIAL, LONG-TERM

Vala Assay: #MP2

Cell Type: Human HuH-7 cells, derived from hepatocellular carcinoma

Main Goal of Assay: Assess mitochondrial and cellular health by evaluating mitochondrial membrane potential after 72 hours of exposure to test compounds. During apoptosis, mitochondria lose their membrane potential due to collapse of the H+ gradient across the inner mitochondrial membrane. Tetramethyl rhodamine methyl ester (TMRM) is a cell permeant cationic dye that accumulates in the matrix of healthy mitochondria due to the negative membrane potential. When the membrane potential is lost, TMRM uptake into mitochondria is prevented and the fluorescent signal is lost.

CyteSeer® Data Readout

- Fluorescence channel #1 (Hoescht): cell number, nuclear size, ploidy, apoptotic fragmentation
- Fluorescence channel #2 (red channel): TMRM (mitochondrial membrane potential)

KINASE ACTIVATION ASSAYS

1. P38-MAPK ACTIVATION

Vala Assay: #P38MPK1

Cell Type: HeLa

Main Goal of Assay: Determine test compound effects on phosphorylation and cellular localization of p38-MAPK (mitogen-activated protein kinase). p38-MAPK activation occurs in response to cell stressors such as oxidative stress, inflammation, ultraviolet radiation, and heat shock. p38-MAPK also participates in cell differentiation, proliferation, tumorigenesis, and metastasis. In response to stimuli, p38-MAPK translocates to the nucleus, where it phosphorylates transcription factors such as FOXO1. This assay provides an integrated readout of compound effects on p38-MAPK-activating signaling pathways within cells. This assay can be multiplexed to measure co-incident activation of another kinase along with p38-MAPK.

CyteSeer® Data Readout

- Fluorescence channel #1 DAPI: cell number, ploidy
- Fluorescence channel #2 (green channel): phospho-p38-MAPK (expression level and nuclear localization)
- Fluorescence channel #3 (red channel): multiplexed staining for another phosphorylated kinase (optional)

2. ERK-MAPK ACTIVATION

Vala Assay: #ERKMPK1

Cell Type: HeLa

Main Goal of Assay: Determine test compound effects on phosphorylation and cellular localization of ERK-MAPK (mitogen-activated protein kinase). ERK-MAPK plays key roles in embryonic development, cardiac function, cell proliferation, and tumorigenesis. In response to stimuli, ERK-MAPK translocates to the nucleus, where it phosphorylates transcription factors such as ELK. This assay provides an integrated readout of compound effects on ERK-MAPK-activating signaling pathways within cells. This assay can be multiplexed to measure co-incident activation of another kinase along with ERK-MAPK.

CyteSeer® Data Readout

- Fluorescence channel #1 DAPI: cell number, ploidy
- Fluorescence channel #2 (green channel): phospho-ERK-MAPK (expression level and nuclear localization)
- Fluorescence channel #3 (red channel): multiplexed staining for another phosphorylated kinase (optional)

3. JNK-MAPK ACTIVATION

Vala Assay: #JNKMPK1

Cell Type: HeLa

Main Goal of Assay: Determine test compound effects on phosphorylation and cellular localization of JNK-MAPK (mitogen-activated protein kinase). JNK-MAPK activation occurs in response to cell stressors such as oxidative stress, inflammation, ultraviolet radiation, and heat shock. JNK-MAPK also participates in cell differentiation, proliferation, tumorigenesis, and metastasis. In response to stimuli, JNK-MAPK translocates to the nucleus, where it phosphorylates transcription factors such as c-Jun. This assay provides an integrated readout of compound effects on JNK-MAPK-activating signaling pathways within cells. This assay can be multiplexed to measure co-incident activation of another kinase along with JNK-MAPK.

CyteSeer® Data Readout

- Fluorescence channel #1 DAPI: cell number, ploidy
- Fluorescence channel #2 (green channel): phospho-JNK-MAPK (expression level and nuclear localization)
- Fluorescence channel #3 (red channel): multiplexed staining for another phosphorylated kinase (optional)