Promega

Sensitive, Fast and Reliable Cell-based Assays for

Cosmetic and Personal Care Product Development and Safety Testing

Contents

The need for alternative methods for cosmetic testing

According to European Union (EU) regulation, all finished cosmetics or personal care products and their ingredients are required to go through safety testing. In the past, safety testing has been done using live animals. This has changed as EU directives and subsequent regulation have encouraged alternative methods, such as cell-based tests. In 2011, the European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM) was established to support the validation and promotion of alternative methods. Their goal is to reduce, refine and replace animal testing. In 2013, the EU banned the marketing of cosmetics products that contain animal-tested ingredients. This further emphasizes the need for alternative safety-testing methods that can substitute for animal tests.

How can cell-based assays evaluate product safety?

Cultured cells have been widely used in the lab as substitutes for live animals. This is possible because the effect of a test compound on cultured cells is often similar to its effect on a live animal. In vitro cell-based assays can thus be used to evaluate the safety of finished cosmetics or personal care products and the raw materials they are made from. For example, compounds that induce cytotoxicity in vitro are likely cytotoxic in vivo, depending on dose, exposure time and site of application.

Why choose Promega cell-based assays?

Reliable and Sensitive Detection

Our cell-based assays are based primarily on bioluminescence, but we also incorporate fluorescence for its specific enabling features. While bioluminescence provides high sensitivity and wide dynamic range, fluorescence enables multiple color outputs for all-fluorescent or fluorescent/bioluminescent multiplex assays. Our assay chemistries typically produce stable signals for ease of use.

Complete Portfolio of Assays and Instruments

We offer assays for viability, cell death, apoptosis, inflammation, oxidative or chemical stress, energy metabolism, epigenetics and cell signaling. The assays can be used for either end-point or real-time analysis and can be applied to single samples or 96-, 384- and 1536-well plate formats. Most of our assays can be combined in multiplex format to measure two or more parameters from a single sample. We also provide instrumentation for reading single samples or multiwell plates in bioluminescent, fluorescent or multiplex modes.

Fast and Simple Protocols

Our cell-based assays have simple “add-mix-read” protocols (Figure 1). Just add reagent to sample well, incubate, then measure bioluminescence or fluorescence using a plate-reader. Data analysis is easily done by graphing the average signal values of sample wells.

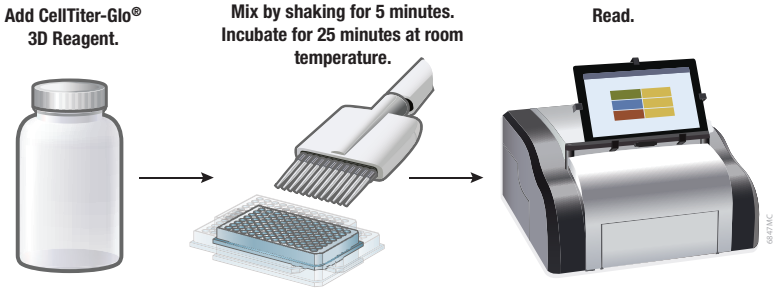
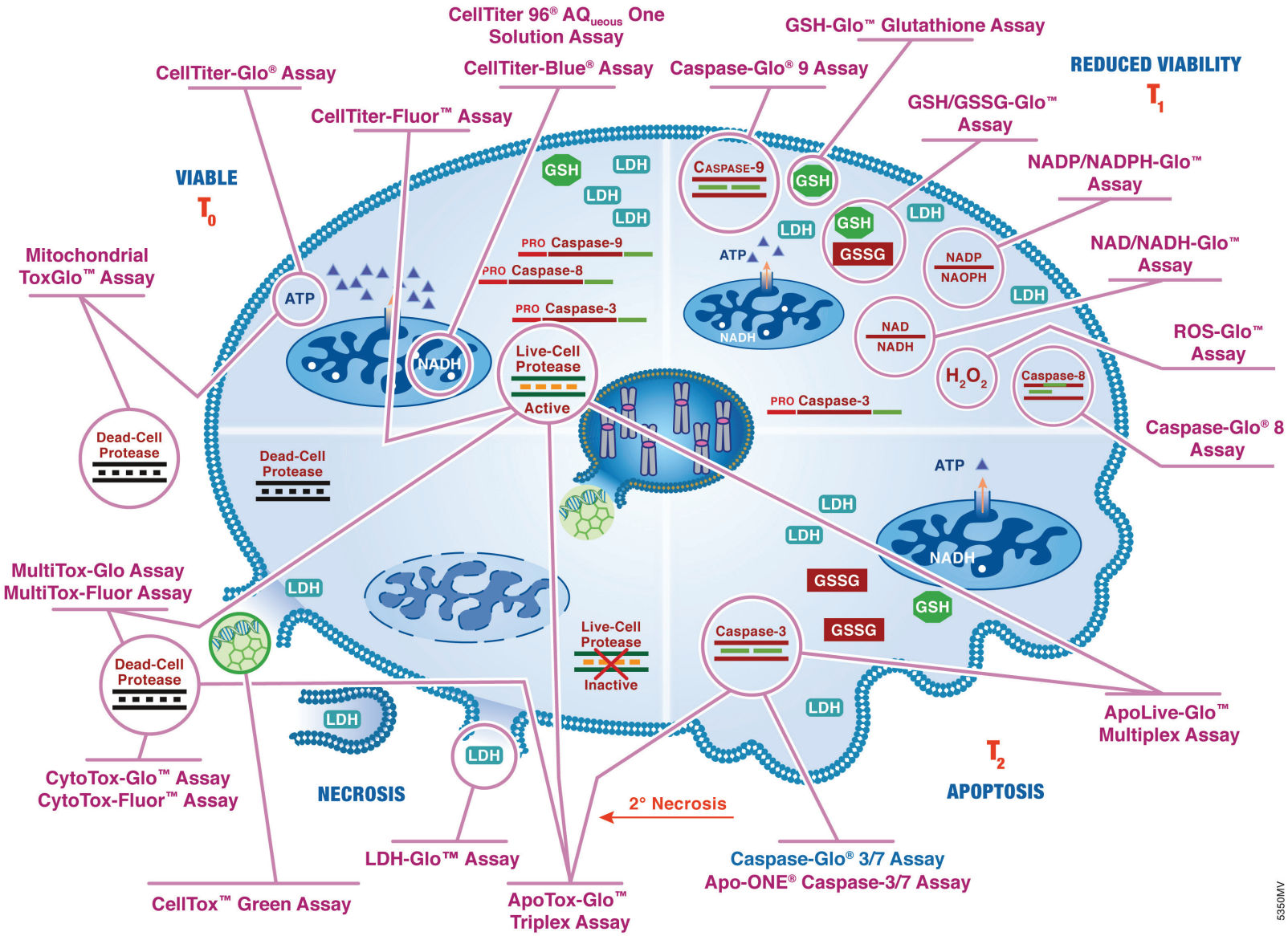


Figure 1. Add-mix-read protocol for the CellTiter-Glo® 3D Cell Viability Assay.

Promega Cell-Based Assays



Cell Health Assays: Viability, Cytotoxicity and Apoptosis

Cytotoxic compounds may harm or kill cells through different pathways. Some toxins disrupt membranes directly and cause a form of cell death known as primary necrosis. Others act indirectly by inducing apoptosis (programmed cell death), which eventually leads to membrane integrity loss via secondary necrosis. Toxins may also harm cells by inducing oxidative or chemical stress, activating inflammatory pathways, and affecting energy metabolism. Our broad portfolio of cell health assays can reveal the effect of cytotoxic compounds, and can be used to evaluate the safety of cosmetic, skin care or fragrance products and their ingredients.

Cell Viability Assays

Viability assays measure the number of live cells by detecting biomarkers that decline upon cell death. An increase of the viability marker indicates cell proliferation, while a decrease indicates cell death. Viability assays can be multiplexed with cytotoxicity assays for a more definitive analysis of cell growth or death caused by cosmetic compounds. Our cell viability assays (Table 1) measure the following markers:

ATP: ATP is the main energy currency of living cells. ATP levels correlate with the number of viable cells and rapidly decline upon cell death. ATP can be detected using ATP-dependent bioluminescent light production by firefly luciferase (Figure 2).

Reducing capacity: The intracellular milieu of a live cell is a reducing environment in which reducing capacity is lost upon cell death. Therefore, viability can be measured by the reduction of specific probes.

Live-cell protease: This is a unique intracellular protease activity that is rapidly lost upon cell death. It works as a marker of viable cells when paired with a selective cell-permeable fluorogenic substrate.

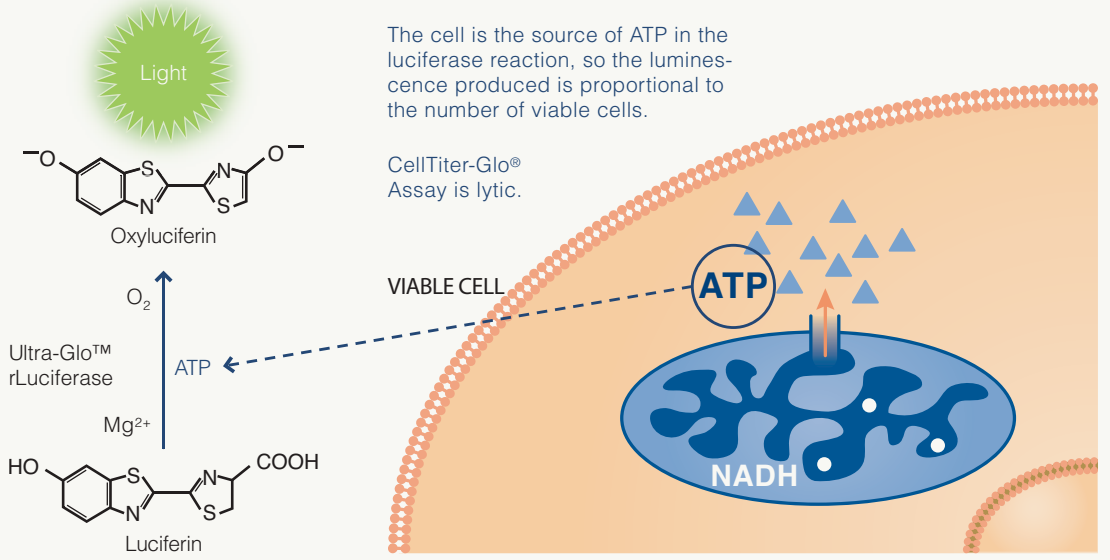


Figure 2. CellTiter-Glo® Luminescent Cell Viability Assay Overview

Table 1: Viability Assays

Assay	Marker	Assay Time/ Sensitivity	Readout	Description	Features
CellTiter-Glo®	ATP	10 minutes/10 cells	Luminescence	• 1 addition • Add & read	Measures ATP in live cells
CellTiter-Glo® 2.0	ATP	10 minutes/10 cells	Luminescence	• 1 addition • Add & read	• Measures ATP in live cells • Longer storage stability
CellTiter-Glo® 3D	ATP	10 minutes/10 cells	Luminescence	• 1 addition • Add & read	• Measures ATP in live cells • Optimized for 3D cultures
BacTiter-Glo™	ATP	5 minutes/10 cells	Luminescence	• 1 addition • Add & read	• Measures ATP in live cells • Optimized for prokaryotic cells
CellTiter-Fluor™	Intercellular live-cell protease	0.5–3 hours/ 400 cells	Fluorescence $400_{Ex}/505_{Em}$	• 1 addition • Add-incubate-read	Measures live-cell protease activity
RealTime-Glo™ MT	Luciferase pro-substrate conversion	Kinetic 0.5–72 hours/<100 cells	Luminescence	• 1 addition • Add & read repeatedly	• Measures reducing capacity of live cells • Monitors time course for up to 72 hours
CellTiter-Blue®	Fluorogenic dye reduction	1–4 hours/100 cells	Fluorescence $560_{Ex}/590_{Em}$	• 1 addition • Add-incubate-read	Measures reducing capacity of live cells
CellTiter 96® Non-radioactive Cell Proliferation	Colorimetric dye reduction	1–4 hours/1000 cells	Absorbance 570nm	• 1 addition • Requires solubilization step	Measures reducing capacity of live cells
CellTiter 96® Aqueous One Solution	Colorimetric dye reduction	1–4 hours/ <1000 cells	Absorbance 490nm	• 1 addition • Add-incubate-read	• Measures reducing capacity of live cells • Fewer steps than MTT assay

For more information about our Cell Health Assays, visit **www.promega.com/CellHealthAssays**

Cytotoxicity Assays

The hallmark of cell death is loss of plasma membrane integrity. Therefore, cytotoxicity assays can detect cell death using markers that are accessible after plasma membrane damage caused by cosmetic compounds. Our cytotoxicity assays (Table 2) measure one of the following markers:

Lactate dehydrogenase (LDH):
When membrane integrity is lost, LDH leaks out of cells into the culture medium where its enzymatic activity is measured as a marker of cell death.

Dead-cell protease:
This is a unique intracellular protease that leaks out of dead cells and remains active in culture medium. The dead-cell protease activity in medium is measured using cell-impermeable substrates that are either fluorogenic or luminogenic.

Cell-impermeable DNA dye:
This is a proprietary dye that is excluded from viable cells, but enters dead cells through damaged membranes to stain dead-cell DNA (Figure 3). Fluorescence from the dye is substantially enhanced when it binds DNA.

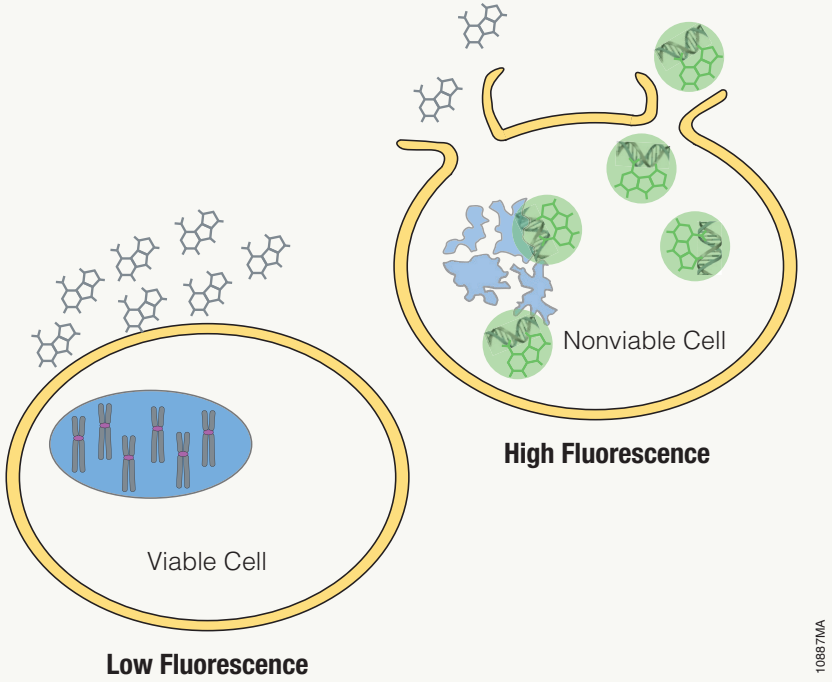


Figure 3. CellTox™ Green Cytotoxicity Assay Overview

Table 2: Cytotoxicity Assays

Assay	Marker	Assay Time/ Sensitivity	Readout	Description	Features
CellTox™ Green (Real-time assay)	DNA binding by cell-impermeable dye	Kinetic 0.5–72 hours/ Plate assay: >100 cells Imaging: 1 cell	Fluorescence $485–500_{Ex}$/ $520–530_{Em}$	• 1 addition • Add & read	• Detects membrane integrity loss due to cell death • Use for time course studies up to 72 hours
CytoTox-Glo™	Dead-cell protease release	15 minutes/10 cells	Luminescence	• 1 addition • Add & read	Detects membrane integrity loss due to cell death
CytoTox-Fluor™	Dead-cell protease release	0.5–3 hours/10 cells	Fluorescence $485_{Ex}/520_{Em}$	• 1 addition • Add & read	• Detects membrane integrity loss due to cell death
LDH-Glo™	LDH release	30 minutes/10 cells	Luminescence	• Sample medium, mix & read	• Detects membrane integrity loss due to cell death • Repeated sampling over time course • Ideal for 3D cultures
CytoTox-One™	LDH release	10 minutes/200 cells	Fluorescence $560_{Ex}/590_{Em}$	• 2 additions • Add & read	• Detects membrane integrity loss due to cell death
ApoTox-Glo™ Triplex	Live-cell protease + dead-cell protease + caspase 3/7	0.5–3 hours/20 cells	Fluorescence & Luminescence	• 2 additions • 3 reads	• Measures viability, cytotoxicity and apoptosis in one assay
ApoLive-Glo™ Multiplex	Live-cell protease + caspase 3/7	0.5–3 hours/20 cells	Fluorescence & Luminescence	• 2 additions • 3 reads	• Measures viability and apoptosis in one assay

For more information about our Cell Health Assays, visit **www.promega.com/CellHealthAssays**

Apoptosis Assays

Apoptosis is a form of programmed cell death, and can be observed by measuring the appearance of various markers after cosmetic compound treatment. Our apoptosis assays (Table 3) measure the following markers:

- Cysteine aspartic acid-specific proteases (caspases).
- Annexin-V binding to phosphatidylserine exposure on the outer leaflet of the plasma membrane (Figure 4).
- Nuclear DNA fragmentation.

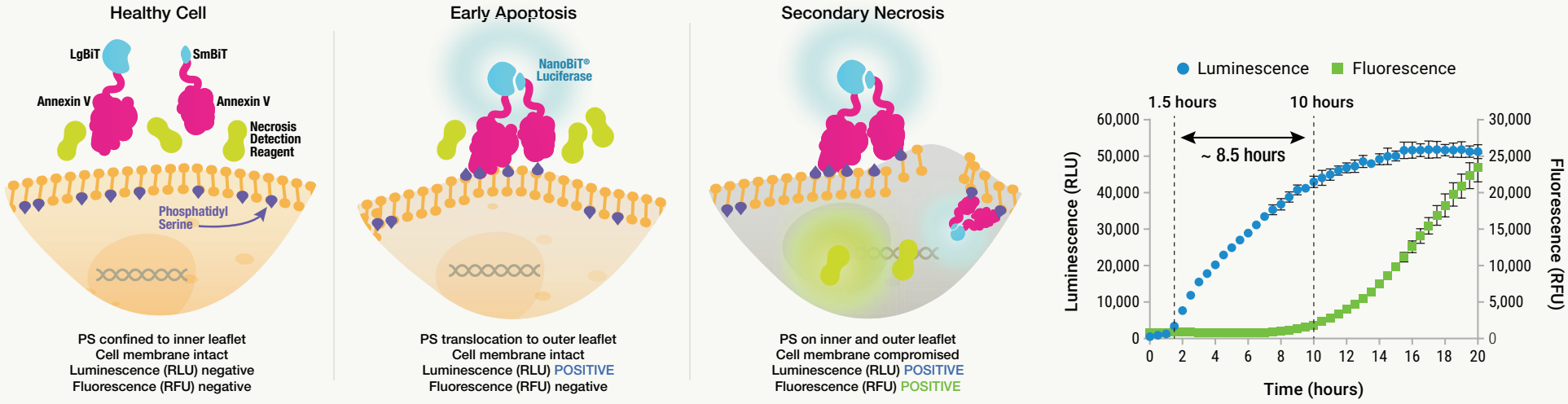


Figure 4. RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay Overview and Example Data. The time delay between the emergence of PS:Annexin V binding and the loss of membrane integrity indicates an apoptotic phenotype that leads to secondary necrosis.

Table 3: Apoptosis Assays

Assay	Marker	Assay Time/ Sensitivity	Readout	Description	Features
RealTime-Glo™ Annexin V Apoptosis & Necrosis	Cell surface Annexin V binding + DNA-binding dye	Kinetic 0.5–48 hours/ Plate assay: >100 cells	Luminescence & Fluorescence	• 1 addition • Add & read	• Measures early apoptosis and secondary necrosis in one assay • Monitors time course for up to 48 hours
Caspase-Glo® 3/7	Caspase 3/7 activity	0.5–3 hours/ 20 cells	Luminescence	• 1 addition • Add & read	Measures apoptosis execution
Caspase-Glo® 8	Caspase 8 activity	0.5–3 hours/ <1600 cells	Luminescence	• 1 addition • Add & read	Measures extrinsic apoptosis pathway initiation
Caspase-Glo® 9	Caspase 9 activity	0.5 hours/ <1600 cells	Luminescence	• 1 addition • Add & read	Measures intrinsic apoptosis pathway initiation
Apo-ONE® Caspase 3/7	Caspase 3/7 activity	0.5–18 hours/ <700 cells	Fluorescence $488_{Ex}/521_{Em}$	• 1 addition • Add & read	Measures apoptosis execution
ApoTox-Glo™ Triplex	Live-cell protease + dead-cell protease + caspase 3/7	0.5–3 hours/ 20 cells	Fluorescence & Luminescence	• 2 addition • 3 reads	Measures viability, cytotoxicity and apoptosis in one assay
ApoLive-Glo™ Duplex	Live-cell protease + caspase 3/7	0.5–3 hours/ 20 cells	Fluorescence & Luminescence	• 2 addition • 2 reads	Measures viability and apoptosis in one assay
DeadEnd™ Fluorometric TUNEL System	Nuclear DNA fragmentation	3–4 hours/ single cell	Fluorescence	Multi-step protocol	Labels apoptotic cells for imaging or flow cytometry

For more information about our Cell Health Assays, visit **www.promega.com/CellHealthAssays**

Cytochrome P450 Assays

Cytochrome P450 enzymes (CYPs) catalyze the metabolism of endogenous molecules, including steroid hormones, and exogenous molecules, such as cosmetic compounds that are absorbed through the skin.

While CYP metabolism generally helps eliminate exogenous molecules, in some cases, it activates their toxicity or carcinogenicity. In addition, exogenous molecules, such as cosmetic compounds, may induce CYP genes and enhance elimination or toxic activation of co-administered molecules. We provide a series of bioluminescent assays to determine whether a cosmetic compound induces or inhibits CYP activity (Table 4).

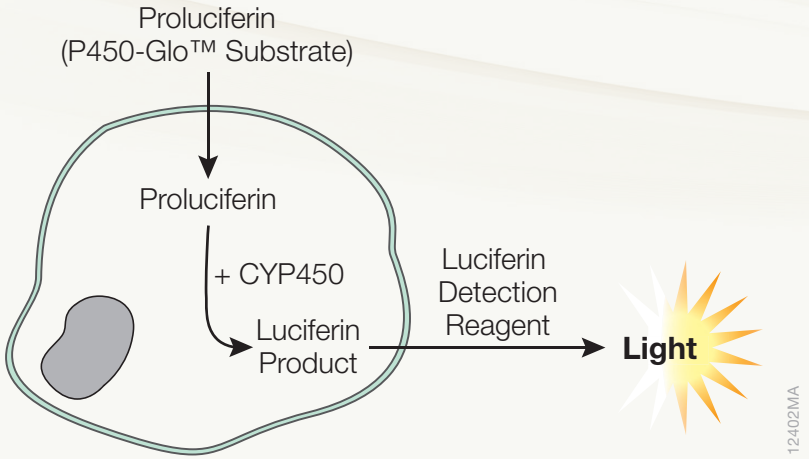


Figure 5. P450-Glo™ Assay Principle

Table 4: Cytochrome P450 Assays

Assay	Marker	Readout	Description	Features
P450-Glo™ CYP3A4 Assay with Luciferin-IPA	Luciferin-IPA oxidation	Luminescence	• 2 additions • Add & read	Measures CYP3A4 activity in cells
P450-Glo™ CYP1A2 Assay with Luciferin-1A2	Probe substrate dealkylation	Luminescence	• 2 additions • Add & read	Measures CYP1A2 activity in cells
P450-Glo™ CYP1A1 Selective Assay with Luciferin-1A1	Probe substrate dealkylation	Luminescence	• 2 additions • Add & read	Measures CYP1A1 activity in cells (Available only on a custom basis)
P450-Glo™ CYP1A1/1B1 Assay with Luciferin-CEE	Probe substrate dealkylation	Luminescence	• 2 additions • Add & read	Measures CYP1A1/1B1 activity in cells
P450-Glo™ CYP2B6 Assay with Luciferin-2B6	Probe substrate oxidation	Luminescence	• 2 additions • Add & read	Measures CYP2B6 activity in cells
P450-Glo™ CYP2C9 Assay with Luciferin-H	Probe substrate hydroxylation	Luminescence	• 2 additions • Add & read	Measures CYP2C9 activity in cells
P450-Glo™ Biochemical Assays	Probe substrate activation	Luminescence	• 2 additions • Add & read	• Measures CYP activity in cell-free reactions • Available for CYP1A1, -1A2, -1B1, -2B6, -2C8, -2C9, -2C19, -2D6, -2J2 -3A4, -3A5, -3A7, -4A, -4F and others on custom basis.

For more information about our Cell Health Assays, visit **www.promega.com/CellHealthAssays**

Energy Metabolism Assays

To fuel homeostasis and proliferation, cells use a network of regulated energy metabolism pathways that derive energy from nutrients. These energy metabolism pathways undergo reprogramming in response to external and internal signals, and play vital roles in proper cell function and disease states. Metabolite levels provide valuable information about pathway activity. Our bioluminescent assays provide a rapid method for measuring specific metabolites, such as glucose and glutamate, and can be used to detect metabolic changes caused by cosmetic compounds (Table 5).

Table 5: Energy Metabolism Assays

Assay	Marker	Assay Time/ Sensitivity	Readout	Description	Features
NAD(P)H-Glo™	NADH NADPH	30–60 minutes/ ≤0.1µM NAD(P)H	Luminescence	• 1 addition, • Add & read	Measures changes in energy metabolism and oxidative stress
NAD/NADH-Glo™	NAD NADH	30–60 minutes/10nM NAD/NADH, 500 cells	Luminescence	• 1 addition • Add & read	Measures changes in energy metabolism and oxidative stress
NADP/NADPH-Glo™	NADH NADPH	30–60 minutes/10nM NADP/NADPH, 500 cells	Luminescence	• 1 addition • Add & read	Measures changes in energy metabolism and oxidative stress
Glucose Uptake-Glo™	2-deoxyglucose-6-P accumulation	60 minutes/0.5µM 2DG6P, <1000 cells	Luminescence	• 1 addition • Add & read	Measures glucose uptake in cells
Glucose-Glo™	Glucose	60 minutes/30nM glucose	Luminescence	• 1 addition • Add & read	Measures changes in glycolysis or glucose production during gluconeogenesis
Lactate-Glo™	Lactate	60 minutes/100nM lactate	Luminescence	• 2 additions • 3 reads	Measures changes in glycolysis
Glutamate-Glo™	Glutamate	60 minutes/10nM glutamate	Luminescence	• 2 additions • 2 reads	Measures changes in glutaminolysis
Glutamine/ Glutamate-Glo™	Glutamine Glutamate	60 minutes/20nM glutamate or glutamate	Luminescence	Homogeneous, add-only protocol	Measures changes in glutaminolysis

For more information about our Energy Metabolism Assays, visit **www.promega.com/EnergyMetabolismAssays**

Oxidative and Chemical Stress Assays

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the capacity to eliminate ROS and repair oxidative damage. Oxidative stress can be induced by cosmetic compounds that are chemical oxidants, inhibitors of antioxidant activities or uncouplers of mitochondrial function.

Direct markers of oxidative stress include a low ratio of reduced to oxidized glutathione (GSH/GSSG), reduced ATP levels and increased H_2O_2 levels (Table 5). H_2O_2 levels can often be used to represent ROS levels because other ROS are rapidly converted to H_2O_2. Besides direct markers, indirect induction of certain transcription regulators can also indicate activation of oxidative and chemical stress responses. Induction of these regulators can be monitored in specific cell types using luciferase reporter vectors that contain stress response element-driven promoters (Tables 7 and 8).

Table 6: Oxidative Stress Assays

Assay	Marker	Assay Time/ Sensitivity	Readout	Description	Features
ROS-Glo™ H_2O_2	H_2O_2	20 minutes/10nM H_2O_2, 5,000 cells	Luminescence	• 1 addition • Add & read	Measures H_2O_2 directly in cell culture or in defined enzyme reactions
GSH-Glo™ Glutathione	Reduced glutathione (GSH)	45 minutes/80nM GSH, 300–1000 cells	Luminescence	• 2 additions • Add & read	Measures toxicological response and oxidative stress
GSH/GSSG-Glo™	GSH GSSG	Reduced glutathione (GSH)	Luminescence	• 2 x 3 additions • Add & read	Measures toxicological response and oxidative stress
Mitochondrial Tox-Glo™	ATP and dead-cell protease	45 minutes/2000 cells	Luminescence & Fluorescence	• Multiplex assay • 2 additions, 2 reads	Detects mitochondrial dysfunction

For more information about our Oxidative and Chemical Stress Assays, visit **www.promega.com/OxidativeStressAssays**

Table 7: Stress Signaling Pathway Luciferase Vectors

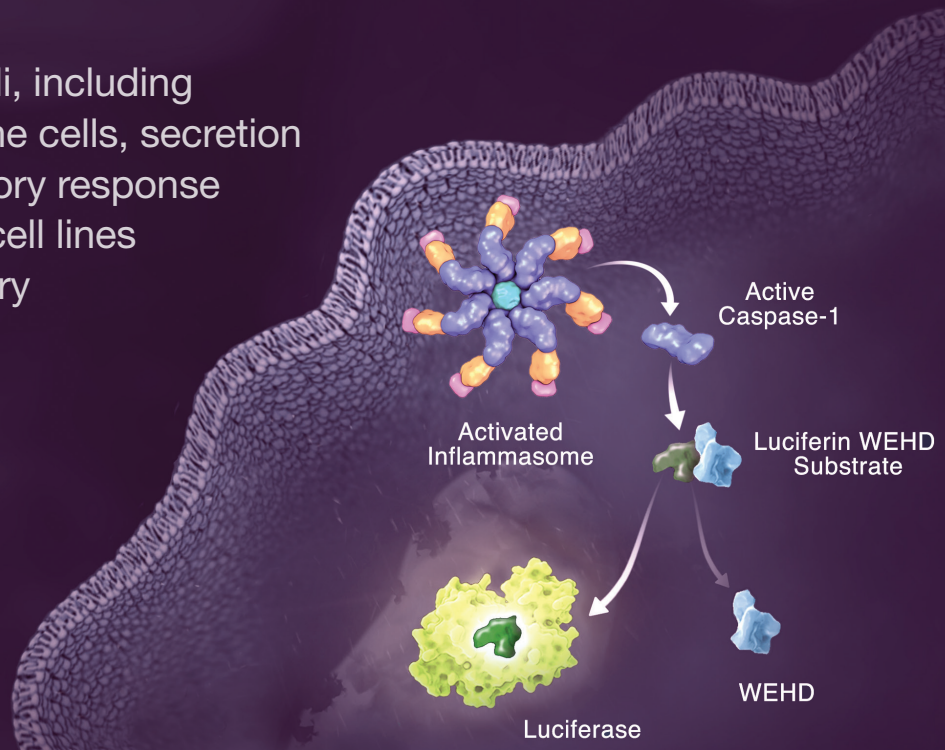
Tox Pathway	Transcription Regulator	Response Element	Luciferase	Vector
DNA damage	p53	p53 RE	Firefly or NanoLuc or Renilla	• pGL4.38[luc2P/p53-RE/Hygro] • pNL[NLucP/p53-RE/Hygro] • pGL4[hRlucP/p53-RE/Hygro]
Hypoxia	Hif1α	HRE	Firefly	pGL4.42[luc2P/HRE/Hygro]
Antioxidant response	Nrf2	ARE	Firefly or NanoLuc	• pGL4.37[luc2P/ARE/Hygro] • pNL[NlucP/ARE/Hygro]
Heat shock	Hsf1	HSE	Firefly	pGL4.41[luc2P/HSE/Hygro]
ER stress	ATF6	ATF6 RE	Firefly	pGL4.39[luc2P/ATF6 RE/Hygro]
Heavy metal stress	Mtf1	MRE	Firefly or NanoLuc	• pGL4.40[luc2P/MRE/Hygro] • pNL[NlucP/MRE/Hygro]
Xenobiotic stress	AhR	AhRE	Firefly or NanoLuc	• pGL4.43[luc2P/XRE/Hygro] • pNL[NlucP/XRE/Hygro]

Table 8: Luciferase Reporter Assay Detection Assays

Assay System	Assay Time	Brightness	Luciferase	Description
Steady-Glo®	10 minutes	+	Firefly	add-mix-read
Bright-Glo™	5 minutes	+ + +	Firefly	add-mix-read
One-Glo™	5 minutes	+ +	Firefly	add-mix-read
Renilla-Glo™	10 minutes	+ +	*Renilla*	add-mix-read
Nano-Glo®	5 minutes	+ + + +	NanoLuc	add-mix-read

Inflammation: Assays, Reporter Vectors and Cell Lines

Inflammation is a protective response to harmful stimuli, including cosmetic compounds. It involves recruitment of immune cells, secretion of inflammatory cytokines, and activation of inflammatory response signaling pathways. Our assays, reporter vectors and cell lines can be used to detect inflammatory or anti-inflammatory effects of cosmetic compounds.



Inflammasome Assay

Innate immune cells respond to harmful stimuli with the formation of protein complexes called inflammasomes, which leads to caspase-1 activation. Caspase-1 activation stimulates processing and release of the cytokines IL-1 and IL-18, eventually causing pyroptosis, an immunogenic form of programmed cell death. We offer an inflammasome assay to monitor the effects of compounds on caspase 1 activation (Table 9).

Table 9: Inflammasome Assay

Assay	Marker	Assay Time/ Sensitivity	Readout	Description	Features
Caspase-Glo® 1 Inflammasome Assay	Caspase 1 induction/ inflammasome activation	1 hour/10,000 cells	Luminescence	• 1 addition • Add & read	Measures innate immune response to pathogens and other danger signals

For more information about our Inflammation Assays, visit **www.promega.com/InflammationAssays**

Cytokine reporter vectors and cell lines

Cytokines are small signaling molecules that activate immune responses. Reporter cell lines provide a simple, fast and quantitative method to screen for cosmetic compounds that induce or inhibit cytokine activity. We offer a family of luciferase reporter vectors (Table 10) and reporter cell lines (Table 11) to help you detect cellular responses to cytokines.

Table 10: Cytokine Reporter Vectors

Luciferase	Response Element	Description
Firefly	Human IL2 Promoter	pGL4[luc2P/IL-2/Hygro] Vector
Firefly	IFN-γ Activation Site (GAS) Response Element	pGL4[luc2P/GAS-RE/Hygro] Vector
Firefly	Human IL8 Promoter	pGL4[luc2P/hIL8/Hygro] Vector
Firefly	IRF1 Response Element	pGL4[luc2P/STAT4-RE/Hygro] Vector
Firefly	Human IL2 Promoter	pGL4[luc2P/IL-2/Puro] Vector
NanoLuc	Interferon Stimulated Response Element (ISRE)	pNL[NlucP/ISRE/Hygro] Vector
NanoLuc	IFN-γ Activation Site (GAS) Response Element	pNL[NlucP/GAS-RE/Hygro] Vector
Firefly	Sis-inducible Element (SIE) Response Element	pGL4.47[luc2P/SIE/Hygro] Vector
Firefly	Interferon Stimulated Response Element (ISRE)	pGL4.45[luc2P/ISRE/Hygro] Vector

Table 11: Cytokine Reporter Cell Lines

Pathway	Luciferase	Response Element	Host Cell Line	Description
Interleukin Reporter Cells				
IL6	Firefly	SIE	HepG2	GloResponse SIE-luc2P HepG2 Cell Line
IL1	Firefly	hIL8	A549	GloResponse IL8-luc2P Cell Line
IL2	Firefly	STAT5 RE	CTLL-2	CTLL-2 luc2P-STAT5 Cell Line
IL12	Firefly	STAT4 RE	HEK293	HEK293 luc2P-STAT4 Cell Line
IL15	Firefly	STAT5 RE	CTLL-2	CTLL-2 luc2P-STAT5 Cell Line
IL23	Firefly	SIE	HEK293	HEK293 luc2P-SIE Cell Line
Interferon Reporter Cells				
IFNα	Firefly	ISRE	HEK293	HEK293 ISRE-luc2P Cell Line
IFNγ	Firefly	GAS	HepG2	GloResponse GAS-luc2P HepG2 Cell Line
IFNβ	Firefly	ISRE	HEK293	HEK293 ISRE-luc2P Cell Line

Epigenetic Assays

The organization of chromosomes into chromatin involves wrapping of the DNA strands around histone proteins to form nucleosomes. Post-translational modifications, such as acetylation or methylation, of those histone proteins can impact gene expression.

The heritable differences in gene function based on histone protein modification, instead of DNA sequence, is a form of “epigenetic” inheritance. Acetylation of histones is done by acetyltransferases (HATs), which relax histone/DNA binding and permits gene expression, while histone deacetylases (HDACs) promote chromatin compaction and reduce gene expression. Similarly, histone methylation that increases histone/DNA affinity generally reduces gene transcription and vice versa. Protein-protein interactions also play a role in epigenetics. For example, epigenetic factors, such as bromodomain proteins, bind to histone tails and alter gene expression. We provide epigenetic assays (Table 12) that can measure histone modifier activity or detect histone interactions to help you determine the epigenetic effects of cosmetic compounds.

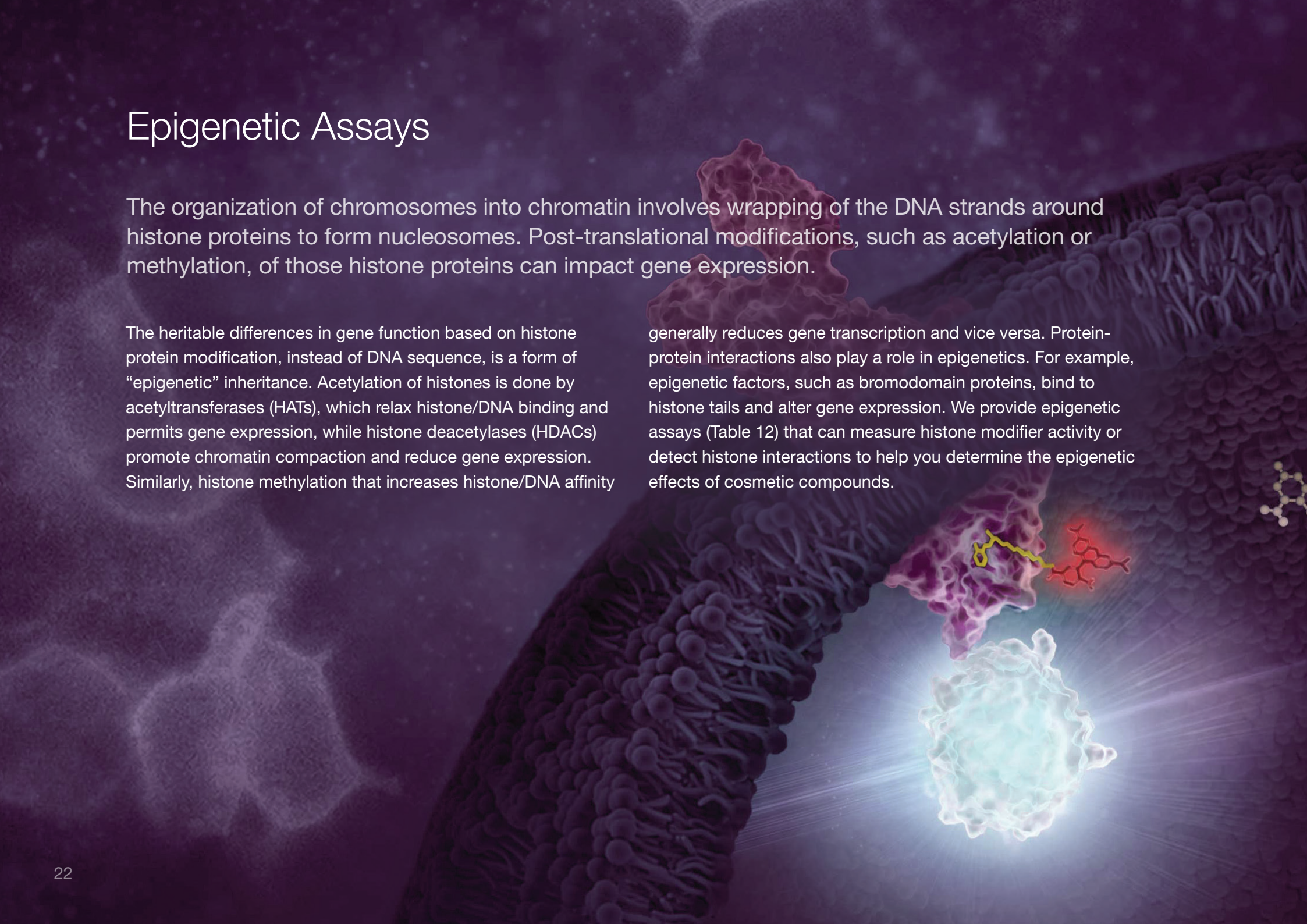


Table 12: Epigenetic Assays

Assay	Marker	Assay Time/ Sensitivity	Readout	Description	Features
HDAC-Glo™ I/II (deacetylase assay)	Probe substrate deacetylation	15–45 minutes/ ≥ 2,000 cells	Luminescence	• 1 addition • Add & read	Measures activity of HDACs class I (1, 2, 3, 8) and class II (4–7, 9, 10)
HDAC-Glo™ IIa (deacetylase assay)	Probe substrate deacetylation	20 minutes/ ≥1,250 cells	Luminescence	• 1 addition • Add & read	Measures activity of HDAC class IIa (4, 5, 7, 9)
HDAC-Glo™ 2 (deacetylase assay)	Probe substrate deacetylation	20 minutes/ ≥1,250 cells	Luminescence	• 1 addition • Add & read	Measures activity of HDAC2
SIRT-Glo™ (deacetylase assay)	Probe substrate deacetylation	20 minutes/ Enzyme-dependent	Luminescence	• 1 addition • Add & read	Measures activity of NAD-dependent class III HDAC (SIRT1–7)
MTase-Glo™ (methyltransferase assay)	SAH	70 minutes/ Enzyme-dependent	Luminescence	• 2 addition • Add & read	Measures activity of any SAM-dependent histone MT enzymes
Succinate-Glo™ Assay (demethylase assay)	Succinate	90 minutes/ Enzyme-dependent	Luminescence	• 2 additions • Add & read	Measures activity of JmjC histone demethylase and Fe(II)/α-ketoglutarate-dependent dioxygenase
NanoBRET™ Bromodomain/Histone Interaction Assays	BRD/histone interactions in live cells	15 minutes/ 200,000 cells per ml	Bioluminescence energy transfer	• Transfect cells • Re-plate cells • Add & read	• Measures interaction between full-length bromodomain (BRD4/BRD9/ BRPF1) and histones (H3.3/H4) • Use in live cells

For more information about our Epigenetic Assays, visit **www.promega.com/EpigeneticAssays**

Cell lines for skin sensitisation testing

Compounds in cosmetics or personal products can potentially cause allergic responses on the skin, known as skin sensitisation. This response involves several key events that take place in the outer layer of the skin (keratinocytes), including inflammatory responses and upregulation of antioxidant/ electrophile response element (ARE)-dependent pathways.

In the past, animal tests were used to determine whether a test compound results in skin sensitisation. Now, animal tests are being replaced by cell-based tests using stable reporter cell lines. The Organisation for Economic Co-operation and Development (OECD) has published Test Guidelines for In Vitro Skin Sensitisation (OECD TG 442D) for two validated luciferase reporter cell lines (KeratinoSens™ and LuSens). These cell lines, when used with the Steady-Glo® or One-Glo™ Luciferase Assay System, can determine skin sensitisation by detecting upregulation of ARE-dependent pathways.

The Test Guideline for In Vitro Skin Sensitisation (OECD TG 442D) is available at: www.oecd.org

KeratinoSens™ or LuSens cell lines are available for purchase at: www.accellerate.me

Genomic solutions for testing compound effects on gene expression

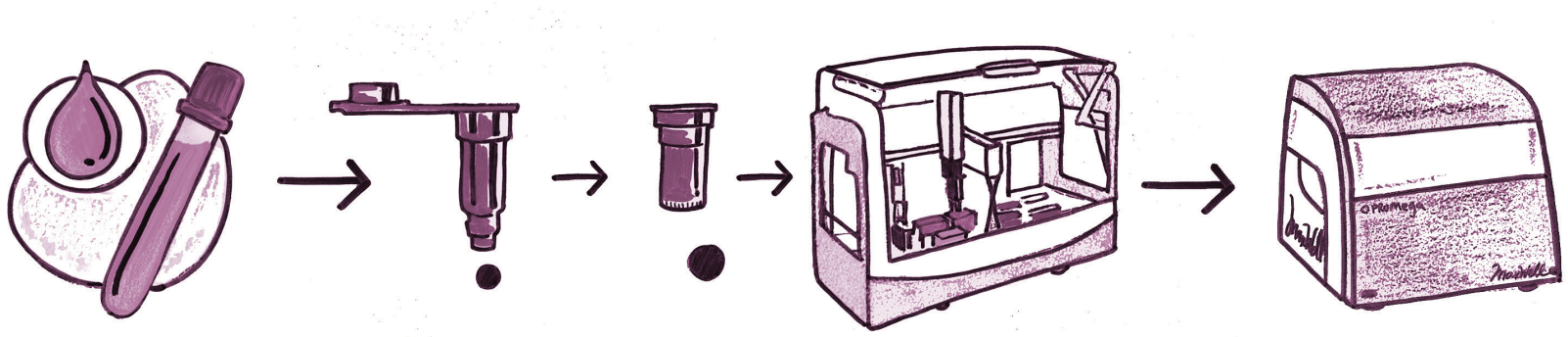
Our menu of genomics offerings includes the latest technology for nucleic acid extraction in multiple throughput formats as well as reagents for amplification and downstream analyses. And, of course, our best-in-class scientific support teams are with you all the way, from helping to set up instrumentation and chemistries to troubleshooting technical results.

Product offerings for Nucleic Acid Extraction include:

- DNA Purification & Quantitation
- RNA Purification & Quantitation
- Benchtop Automation & Purification Systems
- High-Throughput Automation Systems

Product offerings for Amplification include:

- Hot-Start PCR
- Routine PCR
- qPCR and RT-qPCR
- Long PCR



For more information about our genomic products, visit **www.promega.com/GenomicKits**

Instruments for easy assay detection

GloMax® Discover, Explorer and Navigator are state-of-the-art detection instruments offering luminescence, fluorescence and absorbance detection capabilities with preloaded protocols and touch screen functionality. They make data analysis easy.

Easy-To-Use

The intuitive touchscreen display, preloaded protocols and automatic instrument gain adjustments make it simple to produce your data and analyze results.

Integrated with Promega Assays

Optimized, preloaded Promega protocols are part of the GloMax® Systems Software, minimizing the time you spend optimizing instrument settings, and ensuring your experiments run smoothly.

Flexible

Select the detection module or modules you need for your assays. The GloMax® Discover and Explorer Systems can be placed on a bench top for standalone use or integrated into a larger automated platform for higher throughput. Whatever your workflow, GloMax® Systems will accommodate your needs.

Superior Performance

The GloMax® Systems provide a broader dynamic range to measure both high and low samples within the same experiment, resulting in better sensitivity for detecting low-level samples and lower well-to-well cross talk. You can be confident in your readings.

World Class Service and Support

GloMax® Systems come with a comprehensive one-year standard warranty, and our expert service team is available to help if you have questions about either the instrument or chemistries. We offer a full line of additional service products, including Installation and Operation Qualification (IQ/OQ).

To see the full line of GloMax® Instruments, accessories and service products, visit:

www.promega.com/GloMaxInstruments

Promega GmbH
Gutenbergring 10
69190 Walldorf, Germany
www.promega.com

Germany/Austria
Tel: +49 6227 6906-0
Fax: +49 6227 6906-222
de_custserv@promega.com

Poland
Tel: +48 22 531 0667
Fax: +48 22 531 0669
pl_custserv@promega.com