

TECHNICAL BULLETIN

CellTiter-Glo[®] Luminescent Cell Viability Assay

Instructions for Use of Products
G7570, G7571, G7572 and G7573



CellTiter-Glo[®] Luminescent Cell Viability Assay

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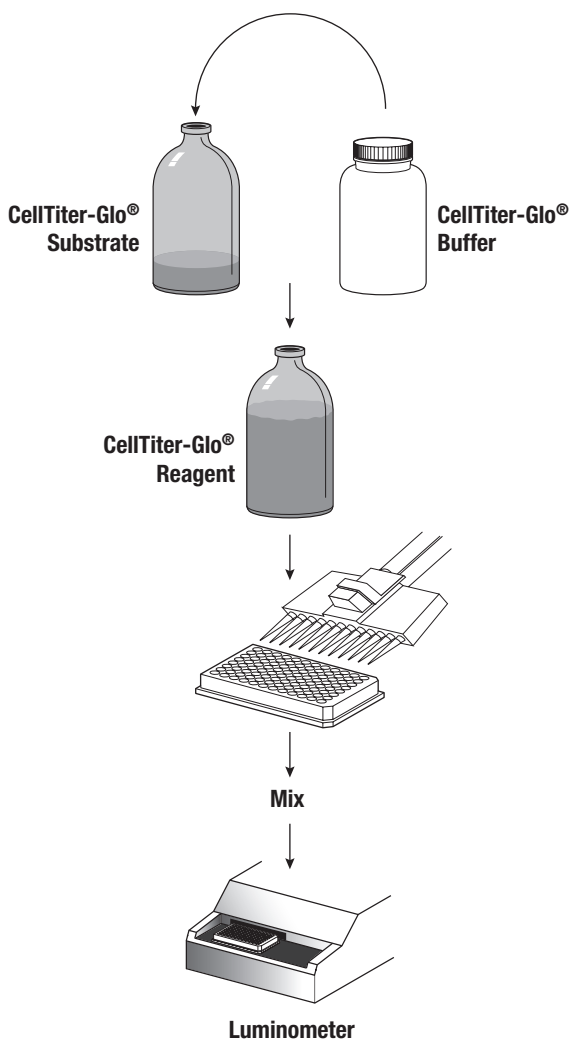
1.	Description.....	1
2.	Product Components and Storage Conditions	5
3.	Performing the CellTiter-Glo [®] Assay.....	6
3.A.	Reagent Preparation	6
3.B.	Protocol for the Cell Viability Assay.....	6
3.C.	Protocol for Generating an ATP Standard Curve (optional)	7
4.	Appendix.....	8
4.A.	Overview of the CellTiter-Glo [®] Assay.....	8
4.B.	Additional Considerations.....	9
4.C.	References	11
4.D.	Related Products	12
5.	Summary of Change	14

1. Description

The CellTiter-Glo[®] Luminescent Cell Viability Assay^(a-d) is a homogeneous method to determine the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. The CellTiter-Glo[®] Assay is designed for use with multiwell-plate formats, making it ideal for automated high-throughput screening (HTS) and cell proliferation and cytotoxicity assays. The homogeneous assay procedure (Figure 1) involves adding a single reagent (CellTiter-Glo[®] Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium or multiple pipetting steps are not required.

The homogeneous “add-mix-measure” format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present (Figure 2). The amount of ATP is directly proportional to the number of cells present in culture in agreement with previous reports (1). The CellTiter-Glo[®] Assay relies on the properties of a proprietary thermostable luciferase (Ultra-Glo[™] Recombinant Luciferase), which generates a stable “glow-type” luminescent signal and improves performance across a wide range of assay conditions. The luciferase reaction for this assay is shown in Figure 3. The half-life of the luminescent signal resulting from this reaction is greater than five hours (Figure 4). This extended half-life eliminates the need for reagent injectors and provides flexibility for continuous or batch-mode processing of multiple plates. The unique homogeneous format reduces pipetting errors that may be introduced during the multiple steps required by other ATP-measurement methods.

1. Description (continued)



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Figure 1. Flow diagram showing preparation and use of CellTiter-Glo® Reagent.

System Advantages

- **Homogeneous:** “Add-mix-measure” format reduces the number of plate-handling steps to fewer than that required for similar ATP assays.
- **Fast:** Data can be recorded 10 minutes after adding reagent.
- **Sensitive:** Measures cells at numbers below the detection limits of standard colorimetric and fluorometric assays.
- **Flexible:** Can be used with various multiwell formats. Data can be recorded by luminometer or CCD camera or imaging device.
- **Robust:** Luminescent signal is very stable, with a half-life >5 hours, depending on cell type and culture medium used.
- **Able to Multiplex:** Can be used with reporter gene assays or other cell-based assays from Promega (2,3).

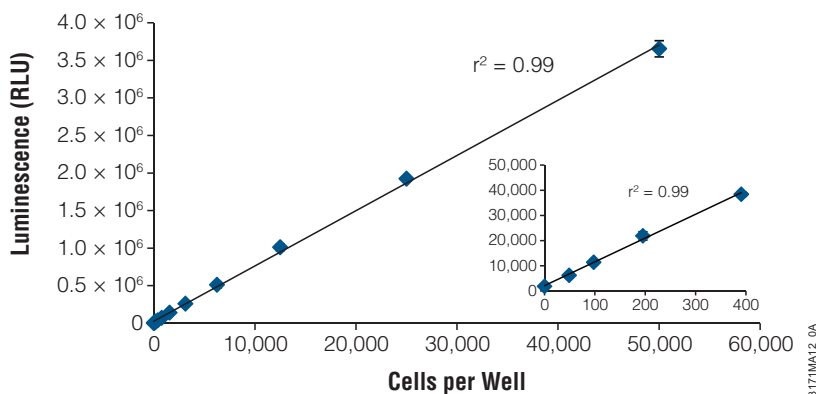


Figure 2. Cell number correlates with luminescent output. A direct relationship exists between luminescence measured with the CellTiter-Glo® Assay and the number of cells in culture over three orders of magnitude. Serial twofold dilutions of HEK293 cells were made in a 96-well plate in DMEM with 10% FBS, and assays were performed as described in Section 3.B. Luminescence was recorded 10 minutes after reagent addition using a GloMax®-Multi+ Detection System. Values represent the mean \pm S.D. of four replicates for each cell number. The luminescent signal from 50 HEK293 cells is greater than three times the background signal from serum-supplemented medium without cells. There is a linear relationship ($r^2 = 0.99$) between the luminescent signal and the number of cells from 0 to 50,000 cells per well.

1. Description (continued)

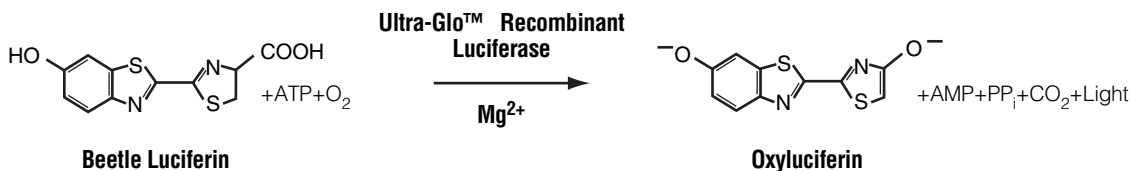


Figure 3. The luciferase reaction. Mono-oxygenation of luciferin is catalyzed by luciferase in the presence of Mg^{2+} , ATP and molecular oxygen.

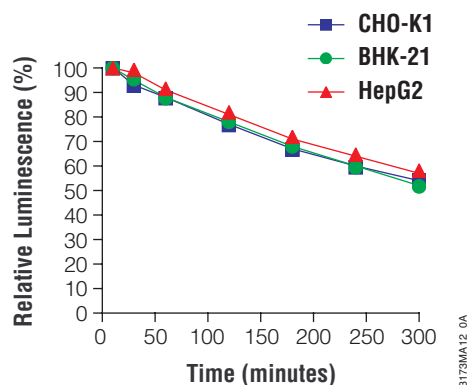


Figure 4. Extended luminescent half-life allows high-throughput batch processing. Signal stability is shown for three common cell lines. HepG2 and BHK-21 cells were grown and assayed in MEM containing 10% FBS, while CHO-K1 cells were grown and assayed in DME/F-12 containing 10% FBS. CHO-K1, BHK-21 and HepG2 cells, at 25,000 cells per well, were added to a 96-well plate. After an equal volume of CellTiter-Glo® Reagent was added, plates were shaken and luminescence monitored over time with the plates held at 22°C. The half-lives of the luminescent signals for the CHO-K1, BHK-21 and HepG2 cells were approximately 5.4, 5.2 and 5.8 hours, respectively.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570

Substrate is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates. Includes:

- 1 × 10ml CellTiter-Glo® Buffer
- 1 vial CellTiter-Glo® Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	10 × 10ml	G7571

Each vial of substrate is sufficient for 100 assays at 100µl/assay in 96-well plates or 400 assays at 25µl/assay in 384-well plates (1,000 to 4,000 total assays). Includes:

- 10 × 10ml CellTiter-Glo® Buffer
- 10 vials CellTiter-Glo® Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	100ml	G7572

Substrate is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates. Includes:

- 1 × 100ml CellTiter-Glo® Buffer
- 1 vial CellTiter-Glo® Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
CellTiter-Glo® Luminescent Cell Viability Assay	10 × 100ml	G7573

Each vial of substrate is sufficient for 1,000 assays at 100µl/assay in 96-well plates or 4,000 assays at 25µl/assay in 384-well plates (10,000 to 40,000 total assays). Includes:

- 10 × 100ml CellTiter-Glo® Buffer
- 10 vials CellTiter-Glo® Substrate (lyophilized)

Storage Conditions: For long-term storage, store the lyophilized CellTiter-Glo® Substrate and CellTiter-Glo® Buffer at –20°C. For frequent use, the CellTiter-Glo® Buffer can be stored at 4°C or room temperature for 48 hours without loss of activity. See product label for expiration date information. Reconstituted CellTiter-Glo® Reagent (Buffer plus Substrate) can be stored at room temperature for up to 8 hours with <10% loss of activity, at 4°C for 48 hours with ~5% loss of activity, at 4°C for 4 days with ~20% loss of activity or at –20°C for 21 weeks with ~3% loss of activity. The reagent is stable for up to ten freeze-thaw cycles, with less than 10% loss of activity.

3. Performing the CellTiter-Glo® Assay

Materials to Be Supplied by the User

- opaque-walled multiwell plates adequate for cell culture
- multichannel pipette or automated pipetting station for reagent delivery
- device (plate shaker) for mixing multiwell plates
- luminometer, CCD camera or imaging device capable of reading multiwell plates
- **optional:** ATP for use in generating a standard curve (Section 3.C)

3.A. Reagent Preparation

1. Thaw the CellTiter-Glo® Buffer, and equilibrate to room temperature prior to use. For convenience the CellTiter-Glo® Buffer may be thawed and stored at room temperature for up to 48 hours prior to use.
2. Equilibrate the lyophilized CellTiter-Glo® Substrate to room temperature prior to use.
3. Transfer the appropriate volume (10ml for Cat.# G7570 and G7571, or 100ml for Cat.# G7572 and G7573) of CellTiter-Glo® Buffer into the amber bottle containing CellTiter-Glo® Substrate to reconstitute the lyophilized enzyme/substrate mixture. This forms the CellTiter-Glo® Reagent.
4. Mix by gently vortexing, swirling or inverting the contents to obtain a homogeneous solution. The CellTiter-Glo® Substrate should go into solution easily in less than 1 minute.

3.B. Protocol for the Cell Viability Assay

We recommend that you perform a titration of your particular cells to determine the optimal number and ensure that you are working within the linear range of the CellTiter-Glo® Assay. Figure 2 provides an example of such a titration of HEK293 cells using 0 to 50,000 cells per well in a 96-well format.

1. Prepare opaque-walled multiwell plates with mammalian cells in culture medium, 100µl per well for 96-well plates or 25µl per well for 384-well plates.



Multiwell plates must be compatible with the luminometer used.

2. Prepare control wells containing medium without cells to obtain a value for background luminescence.
3. Add the test compound to experimental wells, and incubate according to culture protocol.
4. Equilibrate the plate and its contents at room temperature for approximately 30 minutes.

5. Add a volume of CellTiter-Glo® Reagent equal to the volume of cell culture medium present in each well (e.g., add 100µl of reagent to 100µl of medium containing cells for a 96-well plate, or add 25µl of reagent to 25µl of medium containing cells for a 384-well plate).
6. Mix contents for 2 minutes on an orbital shaker to induce cell lysis.
7. Allow the plate to incubate at room temperature for 10 minutes to stabilize luminescent signal.
Note: Uneven luminescent signal within standard plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.
8. Record luminescence.
Note: Instrument settings depend on the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline.

3.C. Protocol for Generating an ATP Standard Curve (optional)

It is a good practice to generate a standard curve using the same plate on which samples are assayed. We recommend ATP disodium salt (Cat.# P1132, Sigma Cat.# A7699 or GE Healthcare Cat.# 27-1006). The ATP standard curve should be generated immediately prior to adding the CellTiter-Glo® Reagent because endogenous ATPase enzymes found in sera may reduce ATP levels.

1. Prepare 1µM ATP in culture medium (100µl of 1µM ATP solution contains 10^{-10} moles ATP).
2. Prepare serial tenfold dilutions of ATP in culture medium (1µM to 10nM; 100µl contains 10^{-10} to 10^{-12} moles of ATP).
3. Prepare a multiwell plate with varying concentrations of ATP standard in 100µl medium (25µl for a 384-well plate).
4. Add a volume of CellTiter-Glo® Reagent equal to the volume of ATP standard present in each well.
5. Mix contents for 2 minutes on an orbital shaker.
6. Allow the plate to incubate at room temperature for 10 minutes to stabilize the luminescent signal.
7. Record luminescence.



4. Appendix

4.A. Overview of the CellTiter-Glo® Assay

The assay system uses the properties of a proprietary thermostable luciferase to enable reaction conditions that generate a stable “glow-type” luminescent signal while simultaneously inhibiting endogenous enzymes released during cell lysis (e.g., ATPases). Release of ATPases will interfere with accurate ATP measurement. Historically, firefly luciferase purified from *Photinus pyralis* (LucPpy) has been used in reagents for ATP assays (1,4–7). However, it has only moderate stability in vitro and is sensitive to its chemical environment, including factors such as pH and detergents, limiting its usefulness for developing a robust homogeneous ATP assay. Promega has successfully developed a stable form of luciferase based on the gene from another firefly, *Photuris pennsylvanica* (LucPpe2), using an approach to select characteristics that improve performance in ATP assays. The unique characteristics of this mutant (LucPpe2^m) enabled design of a homogeneous single-reagent-addition approach to perform ATP assays with cultured cells. Properties of the CellTiter-Glo® Reagent overcome the problems caused by factors, such as ATPases, that interfere with ATP measurement in cell extracts. The reagent is physically robust and provides a sensitive and stable luminescent output.

Sensitivity and Linearity: The ATP-based detection of cells is more sensitive than other methods (8–10). In experiments performed by Promega scientists, the luminescent signal from 50 HEK293 cells is greater than three standard deviations above the background signal from serum-supplemented medium without cells. There is a linear relationship ($r^2 = 0.99$) between the luminescent signal and the number of cells from 0 to 50,000 cells per well in the 96-well format. The luminescence values in Figure 2 were recorded after 10 minutes of incubation at room temperature to stabilize the luminescent signal as described in Section 3.B. Incubation of the same 96-well plate used in the experiment shown in Figure 2 for 360 minutes at room temperature had little effect on the relationship between luminescent signal and number of cells ($r^2 = 0.99$).

Speed: The homogeneous procedure to measure ATP using the CellTiter-Glo® Assay is quicker than other ATP assay methods that require multiple steps to extract ATP and measure luminescence. The CellTiter-Glo® Assay also is faster than other commonly used methods to measure the number of viable cells (such as MTT, alamarBlue® or Calcein-AM) that require prolonged incubation steps to enable the cells’ metabolic machinery to convert indicator molecules into a detectable signal.

4.B. Additional Considerations

Temperature: The intensity and decay rate of the luminescent signal from the CellTiter-Glo® Assay depends on the luciferase reaction rate. Environmental factors that affect the luciferase reaction rate will change the intensity and stability of the luminescent signal. Temperature is one factor that affects the rate of this enzymatic assay and thus the light output. For consistent results, equilibrate assay plates to a constant temperature before performing the assay. Transferring eukaryotic cells from 37°C to room temperature has little effect on ATP content (5). We have demonstrated that removing cultured cells from a 37°C incubator and allowing them to equilibrate to 22°C for 1–2 hours had little effect on ATP content. For batch-mode processing of multiple assay plates, take precautions to ensure complete temperature equilibration. Plates removed from a 37°C incubator and placed in tall stacks at room temperature will require longer equilibration than plates arranged in a single layer. Insufficient equilibration may result in a temperature gradient effect between wells in the center and at the edge of the plates. The temperature gradient pattern also may depend on the position of the plate in the stack.

Chemicals: The chemical environment of the luciferase reaction affects the enzymatic rate and thus luminescence intensity. Differences in luminescence intensity have been observed using different types of culture media and sera. The presence of phenol red in culture medium should have little impact on luminescence output. Assaying 0.1 μM ATP in RPMI medium without phenol red resulted in ~5% increase in luminescence output (in relative light units [RLU]) compared to assays in RPMI containing the standard concentration of phenol red, whereas assays in RPMI medium containing twice the normal concentration of phenol red showed a ~2% decrease in luminescence.

Solvents for the various test compounds may interfere with the luciferase reaction and thus the light output from the assay. Interference with the luciferase reaction can be detected by assaying a parallel set of control wells containing medium without cells. Dimethylsulfoxide (DMSO), commonly used as a vehicle to solubilize organic chemicals, has been tested at final concentrations of up to 2% in the assay and only minimally affects light output.

Plate Recommendations: We recommend using standard opaque-walled multiwell plates suitable for luminescence measurements. Opaque-walled plates with clear bottoms to allow microscopic visualization of cells also may be used; however, these plates will have diminished signal intensity and greater cross talk between wells. Opaque white tape may be used to decrease luminescence loss and cross talk.

Cellular ATP Content: Different cell types have different amounts of ATP, and values reported for the ATP level in cells vary considerably (1,4,11–13). Factors that affect the ATP content of cells may affect the relationship between cell number and luminescence. Anchorage-dependent cells that undergo contact inhibition at high densities may show a change in ATP content per cell at high densities, resulting in a nonlinear relationship between cell number and luminescence. Factors that affect the cytoplasmic volume or physiology of cells also will affect ATP content. For example, oxygen depletion is one factor known to cause a rapid decrease in ATP (1).

Mixing: Optimal assay performance is achieved when the CellTiter-Glo® Reagent is mixed completely with the cultured cells. Suspension cell lines (e.g., Jurkat cells) generally require less mixing to achieve lysis and extract ATP than adherent cells (e.g., L929 cells). Tests were done to evaluate the effect of shaking the plate after adding the CellTiter-Glo® Reagent. Suspension cells cultured in multiwell plates showed only minor differences in light output whether or not the plates were shaken after adding the CellTiter-Glo® Reagent. Adherent cells are more difficult to lyse and show a substantial difference between shaken and nonshaken plates.



4.B. Additional Considerations (continued)

Several additional parameters related to reagent mixing include the force of delivery of CellTiter-Glo® Reagent, sample volume and dimensions of the well. All of these factors may affect assay performance. The degree of reagent mixing required may be affected by the method used to add the CellTiter-Glo® Reagent to the assay plates. Automated pipetting devices using a greater or lesser force of fluid delivery may affect the degree of subsequent mixing required. Complete reagent mixing in 96-well plates should be achieved using orbital plate shaking devices built into many luminometers and the recommended 2-minute shaking time. Special electromagnetic shaking devices that use a radius smaller than the well diameter may be required to efficiently mix contents of 384-well plates. The depth of medium and geometry of the multiwell plates may have an effect on mixing efficiency. We recommend that you take these factors into consideration when performing the assay and empirically determine whether a mixing step is necessary for the individual application.

Luminometers

For highly sensitive luminometric assays, the luminometer model and settings greatly affect the quality of data obtained. Luminometers from different manufacturers will vary in sensitivities and dynamic ranges. We recommend the GloMax® products because these instruments do not require gain adjustments to achieve optimal sensitivity and dynamic range. Additionally, GloMax® instruments are preloaded with Promega protocols for ease of use.

If you are not using a GloMax® luminometer, consult the operating manual for your luminometer to determine the optimal settings. The limits should be verified on each instrument before analysis of experimental samples. The assay should be linear in some portion of the detection range of the instrument used. For an individual luminometer there may be different gain settings. We recommend that you optimize the gain settings.

4.C. References

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2. Farfan, A. *et al.* (2004) Multiplexing homogeneous cell-based assays. *Cell Notes* **10**, 2–5.
3. Riss, T., Moravec, R. and Niles, A. (2005) Selecting cell-based assays for drug discovery screening. *Cell Notes* **13**, 16–21.
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4.D. Related Products

Cell Proliferation Products

Product	Size	Cat.#
ApoLive-Glo™ Multiplex Assay	10ml	G6410
ApoTox-Glo™ Triplex Assay	10ml	G6320
CellTiter-Fluor™ Cell Viability Assay (fluorescent)	10ml	G6080
CellTiter-Blue® Cell Viability Assay (resazurin)	20ml	G8080
CellTiter 96® AQ _{ueous} One Solution Cell Proliferation Assay (MTS, colorimetric)	200 assays	G3582
CellTiter 96® AQ _{ueous} Non-Radioactive Cell Proliferation Assay (MTS, colorimetric)	1,000 assays	G5421
CellTiter 96® AQ _{ueous} MTS Reagent Powder	1g	G1111
CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT, colorimetric)	1,000 assays	G4000

Additional sizes available.

Cytotoxicity Assays

Product	Size	Cat.#
CytoTox-Glo™ Cytotoxicity Assay (luminescent)*	10ml	G9290
Mitochondrial ToxGlo™ Assay*	10ml	G8000
MultiTox-Glo Multiplex Cytotoxicity Assay (luminescent, fluorescent)*	10ml	G9270
MultiTox-Fluor Multiplex Cytotoxicity Assay (fluorescent)*	10ml	G9200
CytoTox-Fluor™ Cytotoxicity Assay (fluorescent)*	10ml	G9260
CytoTox-ONE™ Homogeneous Membrane Integrity Assay (LDH, fluorometric)*	200–800 assays	G7890
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP	1,000–4,000 assays	G7892
CytoTox 96® Non-Radioactive Cytotoxicity Assay (LDH, colorimetric)*	1,000 assays	G1780
GSH-Glo™ Glutathione Assay	10ml	V6911
	50ml	V6912
GSH/GSSG-Glo™ Assay	10ml	V6611
	50ml	V6612

*Additional sizes available.

Luminometers

Product	Size	Cat.#
GloMax [®] -Multi+ Detection System with Instinct [™] Software: Base Instrument with Shaking	1 each	E8032
GloMax [®] -Multi+ Detection System with Instinct [™] Software: Base Instrument with Heating and Shaking	1 each	E9032
GloMax [®] -Multi+ Luminescence Module	1 each	E8041

Apoptosis Products

Product	Size	Cat.#
Caspase-Glo [®] 2 Assay*	10ml	G0940
Caspase-Glo [®] 6 Assay*	10ml	G0970
Caspase-Glo [®] 3/7 Assay*	2.5ml	G8090
Caspase-Glo [®] 8 Assay*	2.5ml	G8200
Caspase-Glo [®] 9 Assay*	2.5ml	G8210
Apo-ONE [®] Homogeneous Caspase-3/7 Assay	1ml	G7792
DeadEnd [™] Fluorometric TUNEL System	60 reactions	G3250
DeadEnd [™] Colorimetric TUNEL System	20 reactions	G7360
Anti-ACTIVE [®] Caspase-3 pAb	50µl	G7481
Anti-PARP p85 Fragment pAb	50µl	G7341
Anti-pS473 Akt pAb	40µl	G7441
Caspase Inhibitor Z-VAD-FMK, 20mM	50µl	G7231
	125µl	G7232

*Additional sizes available.



5. Summary of Change

The following change was made to the 3/15 revision of this document:

Removed an expired patent statement.

^(a)U.S. Pat. Nos. 6,602,677 and 7,241,584, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

^(b)U.S. Pat. No. 7,741,067, Japanese Pat. No. 4485470 and other patents pending.

^(c)U.S. Pat. No. 7,700,310, European Pat. No. 1546374 and other patents pending.

^(d)U.S. Pat. Nos 7,083,911, 7,452,663 and 7,732,128, European Pat. No. 1383914 and Japanese Pat. Nos. 4125600 and 4275715.

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