AUTOMATED PROTOCOL

Automated CellTiter-Glo® Luminescent Cell Viability Assay Protocol

Instructions for Use of Products
G7571, G7572 and G7573

Revised 2/15
EP014

Automated CellTiter-Glo® Luminescent Cell Viability Assay Protocol

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Automated Protocol.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

This document describes automation of the CellTiter-Glo® Luminescent Cell Viability Assay[a,b,c] on the Beckman Coulter Biomek® 2000 and Biomek® FX and Hamilton MICROLAB® STAR automated liquid-handling workstations. For information about available methods, visit: **www.promega.com/automethods/**

Please refer to the *CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin* #TB288 to troubleshoot chemistry issues.

2. Product Components and Storage Conditions

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

Includes:

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

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

Includes:

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

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

Includes:

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

Each 100ml of reagent is sufficient to perform 768 × 100µl assays in 96-well plates or 3,072 × 25µl assays in 384-well plates using the Beckman Coulter Biomek® 2000 workstation. When using the Beckman Coulter Biomek® FX or Hamilton MICROLAB® STAR workstation, 100ml of reagent is sufficient to perform 384 × 100µl assays in 96-well plates or 1,536 × 25µl assays in 384-well plates. The single-plate Biomek® 2000 method includes a 2ml dead volume in the reagent trough, and the single-plate Biomek® FX and Hamilton MICROLAB® STAR methods include a 15ml dead volume in the reagent reservoir. The number of assays processed per 100ml bottle will increase if multiplate methods are run and unused reagent is reused. Do not leave unused reagent at room temperature more than 6–8 hours.

Storage Conditions: Store the CellTiter-Glo® Buffer and CellTiter-Glo® Substrate at –20°C for long-term storage. For frequent use, store the CellTiter-Glo® Buffer at 4°C or at room temperature for 48 hours without loss of activity. Reconstituted CellTiter-Glo® Reagent (Buffer plus Substrate) can be stored at 4°C for 48 hours with approximately 5% loss of activity or at 4°C for 4 days with approximately 20% loss of activity. When stored and handled properly, this system is guaranteed for at least 6 months from the date of purchase.

3. Before You Begin

Materials to Be Supplied by the User

- 96- or 384-well opaque white or black plate suitable for cell culture
- luminometer or CCD camera imaging device capable of reading multiwell plates

3.A. Preparation of Solutions

Please read the following protocol thoroughly before using the CellTiter-Glo® Luminescent Cell Viability Assay. Directions are given for performing the assay in a total volume of 200µl using 96-well plates or in a total volume of 50µl using 384-well plates and a luminescent plate reader. However, the assay can be easily adapted to different volumes provided the 1:1 ratio of CellTiter-Glo® Reagent volume to sample volume is preserved (e.g., 25µl of sample + 25µl of CellTiter-Glo® Reagent).

CellTiter-Glo® Reagent Preparation

1. Thaw the CellTiter-Glo® Buffer, and equilibrate it to room temperature before use. For convenience, the CellTiter-Glo® Buffer may be thawed and stored at room temperature for up to 48 hours before use.

   Note: Temperature affects the rate of the luciferase reaction and thus light output. Equilibrate the Buffer to room temperature before use.

2. Equilibrate the lyophilized CellTiter-Glo® Substrate to room temperature before use.

3. Transfer the volume of CellTiter-Glo® Buffer indicated on the Substrate bottle label into the amber bottle containing the CellTiter-Glo® Substrate to reconstitute the lyophilized substrate. 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 one minute.

3.B. Sample Preparation Before Automated Processing

1. Before starting the assay, prepare the CellTiter-Glo® Reagent as described in Section 3.A and mix thoroughly.

2. Equilibrate the sample to be assayed to room temperature for approximately 30 minutes before performing the assay.

   Note: Transferring eukaryotic cells from 37°C to room temperature has been shown to have little effect on ATP content.

Notes:

1. For best results, empirical determination of the optimal cell number, induction treatment and incubation time for the cell culture system may be necessary.

2. Use identical cell numbers and volumes for the assay and the negative control.

3. Wells that do not contain assay reactions or controls should have a volume of liquid (water or medium) equal to that of the assay and control wells.

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4. Automated Processing Requirements: Beckman Coulter Biomek® 2000 Workstation

4.A. Instrument Requirements for the Biomek® 2000 Workstation

Description	Quantity	Beckman Coulter Part#
Biomek® 2000 Workstation, 50/60 Hz, 100–120V	1	609000
Biomek® 2000 Controller NT	1	609875
BioWorks™ 3.2 for Biomek® 2000	1	609983
MP200 Multichannel Tool	1	609025
Gripper Tool System with disposal option	1	609001
DPC Micromix® 5 Shaker	1	380560
DPC Micromix® 5 Integration kit	1	380561
Tip Rack Holder	1	609121
Gray Labware Holder	2	609120
Frame for Reservoirs	1	372795
Quarter Reservoir	1	372790

4.B. Labware Requirements for the Biomek® 2000 Workstation

Description	Quantity	Ordering Information
Biomek® P250 tips, sterile	1	Beckman Coulter Part# 372655
Costar® 96-well clear-bottom plate, white, polystyrene or equivalent (for 96-well assay)	1	Corning Part# 3610
Costar® 384-well clear-bottom plate, white, polystyrene or equivalent (for 384-well assay)	1	Corning Part# 3707

4.C. Initial Deck Layout for 96-Well Assay on the Biomek® 2000 Workstation

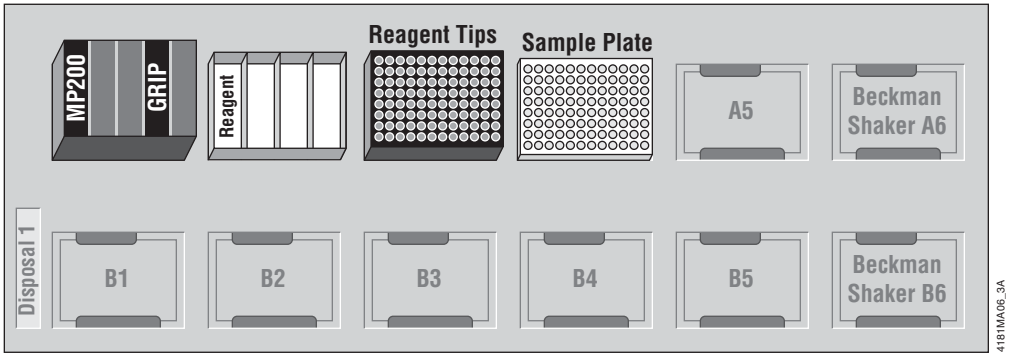

Figure 1. Deck layout for 96-well assay using the CellTiter-Glo® Luminescent Cell Viability Assay on the Biomek® 2000 workstation. This is an example of a CellTiter-Glo® Luminescent Cell Viability 96-well assay deck layout on the Biomek® 2000 workstation.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP200 Multichannel tool; 2) Empty; 3–5) Gripper tool
A2	Frame for Reservoir: Quarter reservoir containing 12ml of CellTiter-Glo® Reagent
A3	Biomek® P250 tips, sterile
A4	96-well assay plate containing 100µl/well of sample, negative control or blank
A5	Empty
A6	Beckman Coulter shaker integration plate holder
B1–B5	Empty
B6	Beckman Coulter shaker integration plate holder

4.D. Initial Deck Layout for 384-Well Assay on the Biomek® 2000 Workstation

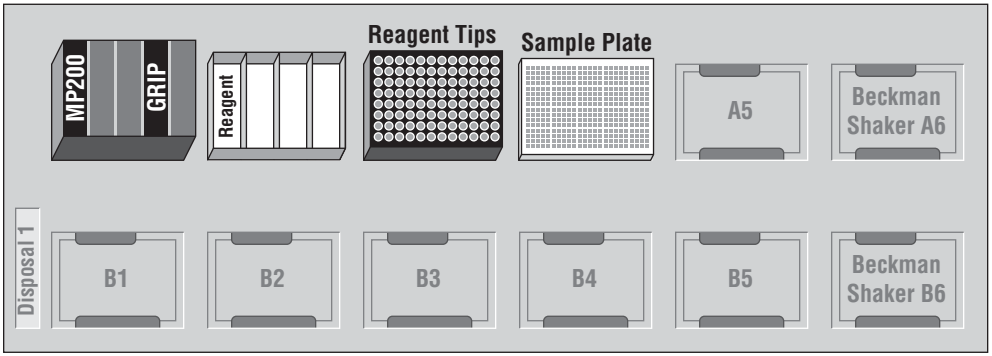

Figure 2. Deck layout for 384-well assay using the CellTiter-Glo® Luminescent Cell Viability Assay on the Biomek® 2000 workstation. This is an example of a CellTiter-Glo® Luminescent Cell Viability 384-well assay deck layout on the Biomek® 2000 workstation.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP200 Multichannel tool; 2) Empty; 3–5) Gripper tool
A2	Frame for Reservoir: Quarter reservoir containing 12ml of CellTiter-Glo® Reagent
A3	Biomek® P250 tips, sterile
A4	384-well assay plate containing 25µl/well of sample, negative control or blank
A5	Empty
A6	Beckman Coulter shaker integration plate holder
B1–B5	Empty
B6	Beckman Coulter shaker integration plate holder

4.E. Pre-Run Biomek® 2000 Workstation-Specific Requirements

Instructions on importing Biomek® 2000 programs, and instructions for integration of the DPC Micromix® 5 Shaker on the Biomek® 2000, are available in the documents: *Importing Biomek® 2000 Programs* and *DPC Micromix® 5 Shaker Integration: Biomek® 2000* (**www.promega.com/automethods/beckman/biomek2000**).

5. Automated Processing Requirements: Beckman Coulter Biomek® FX Workstation

5.A. Instrument Requirements for the Biomek® FX Workstation

Any single-arm multichannel Biomek® FX is able to run this protocol. The protocol also can be adapted for a dual-arm Biomek® FX with at least one multichannel pod.

Part Description	Quantity	Beckman Coulter Part#
Minimum: Biomek® FX Software version 2.1		Contact Beckman Coulter
Minimum number of Labware Positions by 1 POD	2	Contact Beckman Coulter
Tip Loader ALP	1	719356
Orbital Shaker ALP	1	Contact Beckman Coulter
96-channel POD (for 96-well and 384-well assays)	1	Contact Beckman Coulter

5.B. Labware Requirements for the Biomek® FX Workstation

Part Description	Quantity	Ordering Information
Requirements for 96-well assay		
Costar® 96-well clear-bottom plate, white, polystyrene or equivalent	1	Corning Part# 3610
AP96 P250 tips	1	Beckman Coulter Part# 717251
96-well, pyramid-bottom reservoir, polypropylene	1	Innovative Microplate Part# S30014
Requirements for 384-well assay using 96-well tips (96-384-well format)		
Costar® 384-well clear-bottom plate, white, polystyrene or equivalent	1	Corning Part# 3707
AP96 P250 tips	1	BeckmanCoulter Part# 717251
96-well, pyramid-bottom reservoir, polypropylene	1	Innovative Microplate Part# S30014

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5.C. Initial Deck Layout for 96-Well Assay on the Biomek® FX Workstation

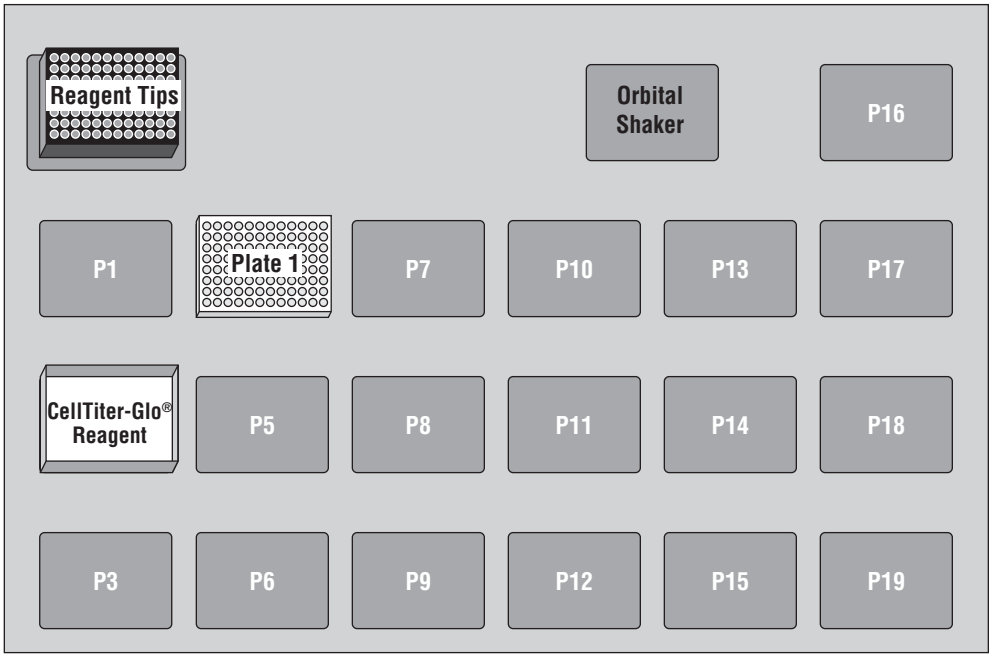

Figure 3. Deck layout for 96-well assay using the CellTiter-Glo® Luminescent Cell Viability Assay on the Biomek® FX workstation. This is an example of a CellTiter-Glo® Luminescent Cell Viability 96-well assay deck layout on the Biomek® FX workstation. Your specific deck layout may be different depending on your Biomek® FX configuration.

ALP Name	Part Sitting on ALP
Tip loader	AP96 P250 tips
P1, P3	Empty
P2	Pyramid-bottom reservoir containing 25ml of CellTiter-Glo® Reagent
P4	96-well assay plate containing 100µl/well of sample, negative control or blank
P5–P19	Empty
Orbital 1	Orbital shaker ALP

5.D. Initial Deck Layout for 384-Well Assay Using a 96-Channel POD on the Biomek® FX Workstation

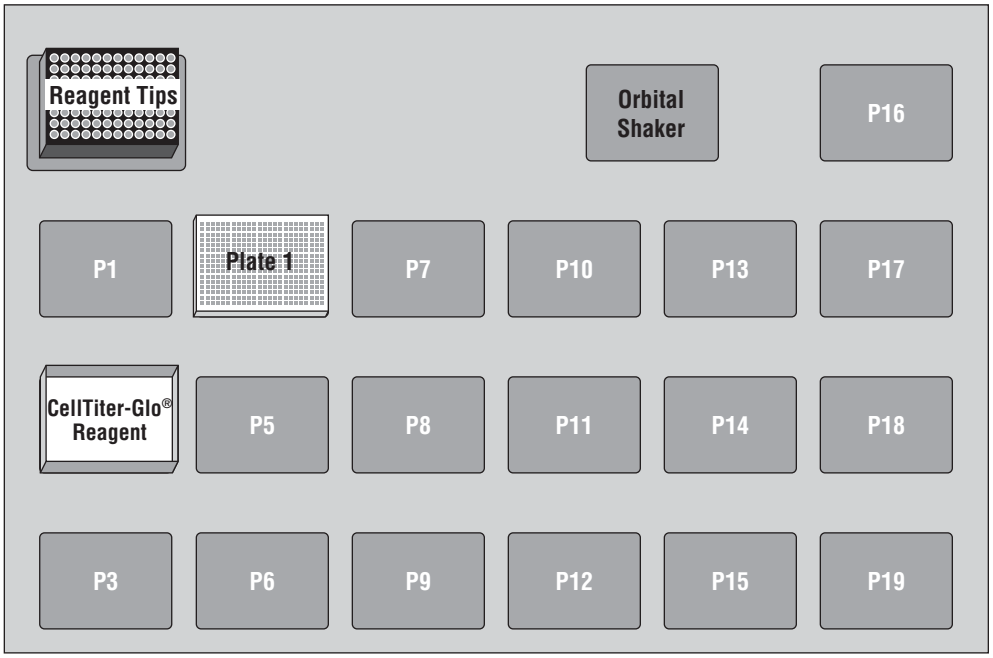

Figure 4. Deck layout for 384-well assay using a 96-channel POD and the CellTiter-Glo® Luminescent Cell Viability Assay on the Biomek® FX workstation. This is an example of a CellTiter-Glo® Luminescent Cell Viability 384-well assay deck layout on the Biomek® FX workstation. Your specific deck layout may be different depending on your Biomek® FX configuration.

ALP Name	Part Sitting on ALP
Tip loader	AP96 P250 tips
P1, P3	Empty
P2	Pyramid-bottom reservoir containing 25ml of CellTiter-Glo® Reagent
P4	384-well assay plate containing 25µl/well of sample, negative control or blank
P5–P19	Empty
Orbital 1	Orbital shaker ALP

5.E. Pre-Run Biomek® FX Workstation-Specific Requirements

The Biomek® FX workstation allows users the flexibility to configure the robot's deck according to need. Because of this flexibility, it is likely that the deck used for writing a Biomek® FX method will differ from an end-user's deck. Therefore, it is generally necessary to map an imported method onto an end-user's deck configuration. To map an imported method onto your deck, please follow the instructions provided in the document: *Biomek® FX Deck Mapping* (**www.promega.com/automethods/beckman/biomekfx/default.asp**).

Prior to the first run of the CellTiter-Glo® Luminescent Cell Viability Assay on the Biomek® FX workstation, it is necessary to ensure that the deck has been properly framed. Failure to do so may result in tips bending during the method and potentially damaging the instrument.

6. Automated Processing Requirements: Hamilton MICROLAB® STAR Workstation

6.A. Instrument Requirements for the MICROLAB® STAR Workstation

The following is a list of Hamilton parts and their corresponding part numbers that are required to automate the CellTiter-Glo® Luminescent Cell Viability Assay on the MICROLAB® STAR workstation.

Part Description	Quantity	Hamilton Part#
MICROLAB® STAR Autoloading liquid-handling workstation with a 96-channel CO-RE Multi-Probe Head and iSWAP labware handling arm	1	Contact Hamilton
DWP Carrier with Shaker (CAT SH10 or Teleshake)	1	Contact Hamilton
5-Position Tip Rack Carrier	1	182085
5-Position Plate Carrier for Standard Microplates	1	182365

6.B. Labware Requirements for the MICROLAB® STAR Workstation

Part Description	Quantity	Part Number
300µl CO-RE Disposable Filter Tips in blue hanging rack	1	Hamilton 235903
96-well Pyramid-Bottom Reservoir (or equivalent)	1	Promega V6801
96-well or 384-well white-walled, clear-bottom assay plate(s)	1–5	Corning 3610 (96-well) Corning 3707 (384-well) (or equivalent)

6.C. Initial Deck Layout for 96-Well Assay on the MICROLAB® STAR Workstation

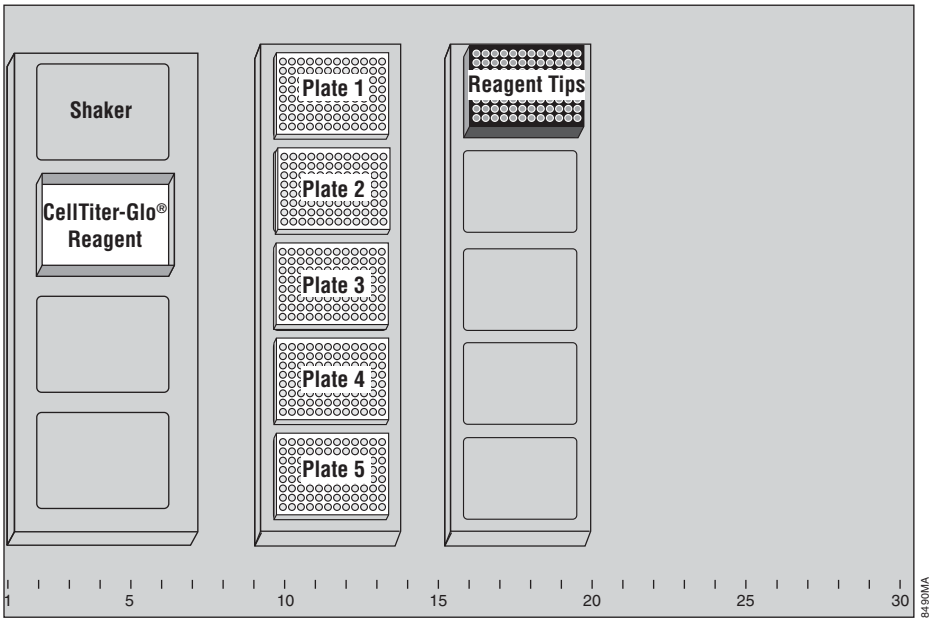

Figure 5. Deck layout for 96-well assay using the CellTiter-Glo® Luminescent Cell Viability Assay on the MICROLAB® STAR workstation. This is an example of a CellTiter-Glo® Luminescent Cell Viability 96-well assay deck layout. Your specific deck layout may be different depending on your MICROLAB® STAR workstation configuration.

Deck Position	Part Name
Grid 1	DWP Carrier with Shaker
Site 1	Shaker Position, no labware
Site 2	96-well Pyramid-Bottom Reservoir (CellTiter-Glo® Reagent)
Grid 8	5-Position Plate Carrier for Standard Microplates
Site 1	96-well white, clear-bottom assay plate
Site 2	96-well white, clear-bottom assay plate (optional)
Site 3	96-well white, clear-bottom assay plate (optional)
Site 4	96-well white, clear-bottom assay plate (optional)
Site 5	96-well white, clear-bottom assay plate (optional)
Grid 18	5-Position Tip Rack Carrier
Site 1	300µl CO-RE Disposable Filter Tips in blue hanging rack

6.D. Initial Deck Layout for 384-Well Assay Using a 96-Probe Head on the MICROLAB® STAR Workstation

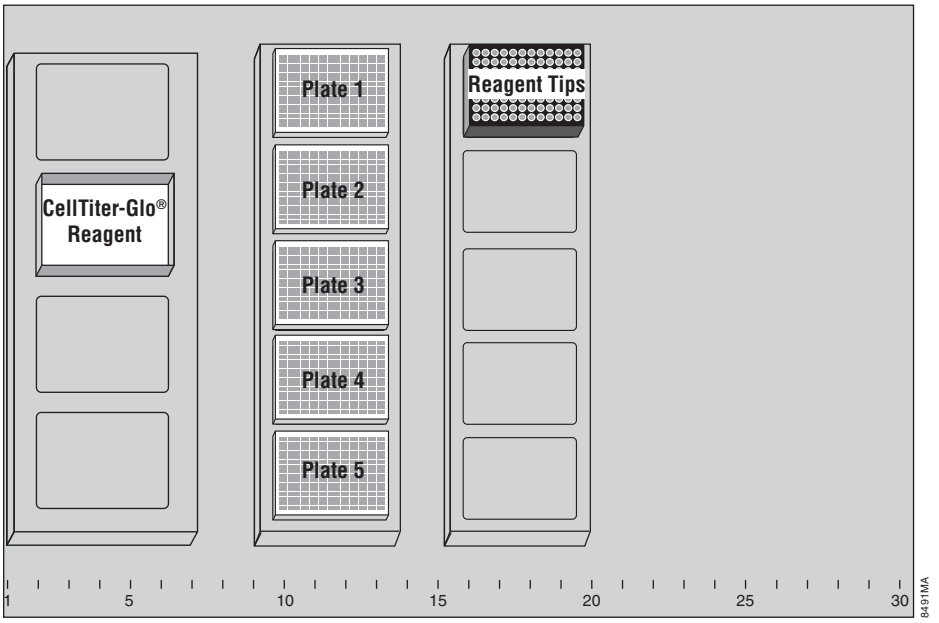

Figure 6. Deck layout for 384-well assay using a 96-Probe Head and the CellTiter-Glo® Luminescent Cell Viability Assay on the MICROLAB® STAR workstation. This is an example of a CellTiter-Glo® Luminescent Cell Viability 384-well assay deck layout. Your specific deck layout may be different depending on your MICROLAB® STAR workstation configuration.

Deck Position	Part Name
Grid 1	DWP Carrier
Site 2	96-well Pyramid-Bottom Reservoir (CellTiter-Glo® Reagent)
Grid 8	5-Position Plate Carrier for Standard Microplates
Site 1	384-well white, clear-bottom assay plate
Site 2	384-well white, clear-bottom assay plate (optional)
Site 3	384-well white, clear-bottom assay plate (optional)
Site 4	384-well white, clear-bottom assay plate (optional)
Site 5	384-well white, clear-bottom assay plate (optional)
Grid 18	5-Position Tip Rack Carrier
Site 1	300µl CO-RE Disposable Filter Tips in blue hanging rack

7. Description of the CellTiter-Glo® Luminescent Cell Viability Assay Method

This overview describes general liquid handling steps required for performing 25µl or 100µl assays in a 384-well or 96-well format. The assay can be adapted to a variety of automated liquid handling robots, as well as to different volumes, as long as the 1:1 ratio of CellTiter-Glo® Reagent:sample volume is preserved. See Section 8 for information on adaptation to liquid handling robots other than those referenced above.

7.A. CellTiter-Glo® Reagent Addition

96-Well Format: CellTiter-Glo® Reagent (100µl) is transferred to the assay plate containing 100µl of blank, untreated control cells or treated cells in culture.

384-Well Format using 96-Channel: CellTiter-Glo® Reagent (25µl) is transferred to the assay plate containing 25µl of blank, untreated control cells or treated cells in culture.

To avoid cross contamination, do not allow the pipette tips to touch the material in the sample wells.

Note: Do not mix CellTiter-Glo® Reagent and samples by pipetting. Mixing in this manner is unnecessary and may create bubbles that interfere with luminescence readings or cross-contaminate the samples. Gentle mixing may be performed using a plate shaker.

7.B. CellTiter-Glo® Reagent and Sample Mix

1. **Assay Plate Transfer.** The assay plate is transferred to the orbital shaker.
2. **Incubation Mix.** The contents of the wells are mixed at 300–500rpm for at least 120 seconds for 96-well plates to induce cell lysis.
3. **Assay Plate Replacement.** The assay plate is transferred back to its original position on the deck.

Incubate sample plate at room temperature for a minimum of 10 minutes. Kinetics may vary depending on cell lines and cell culture media used. Optimal incubation time should be empirically determined. Refer to the product insert for specification for DPBS.

Manually assay samples using a luminescent plate reader after the predetermined incubation time period.

8. General Guidelines for Adaptation to Alternative Robotic Platforms

To avoid cross-contamination of samples, or introduction of bubbles into the wells, ensure that the tips do not touch the liquid in the wells when dispensing the reagent. No tip touches are done on the sides of the wells. This makes it possible to aliquot reagent to more than one plate using a single box of tips.

Do not mix CellTiter-Glo® Reagent and samples by pipetting. Mixing in this manner is unnecessary and may cross-contaminate the samples when using multiplate methods. Gentle mixing may be performed using a plate shaker.

For 384-well plates, dispense near the surface of the liquid to ensure efficient reagent transfer.

9. Summary of Changes

The following changes were made to the 2/15 revision of this document:

1. The patent/license statements were updated.
2. The document design was updated.

[a]U.S. Pat. Nos. 6,602,677, 7,241,584 and 8,030,017, European Pat. No. 1131441, Japanese Pat. Nos. 4537573 and 4520084 and other patents pending.

[b]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.

[c]U.S. Pat. Nos. 7,741,067, 8,361,739, 8,603,767, Japanese Pat. No. 4485470 and other patents pending.

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Biomek is a registered trademark of Beckman Coulter, Inc. BioWorks is a trademark of Beckman Coulter, Inc. Costar is a registered trademark of Corning, Inc. MICROLAB is a registered trademark of Hamilton Company. Micromix is a registered trademark of Clintec Nutrition Co.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.