

Automated CellTiter-Blue™ Cell Viability Assay Protocol

Automated Protocol #EP015

DESCRIPTION OF THE BECKMAN COULTER BIOMEK® 2000 and BIOMEK® FX METHODS WITH PRODUCTS G8081 AND G8082.

Please visit the web site to verify that you are using the most current version of this Automated Protocol.

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I. Description

This describes automation of the CellTiter-Blue™ Cell Viability Assay. Specific instructions are provided for the Beckman Coulter Biomek® 2000 and Biomek® FX. For information about obtaining the validated method, please see:

www.promega.com/automethods/

Please refer to the *CellTiter-Blue™ Cell Viability Assay Technical Bulletin #TB317* to troubleshoot chemistry issues.

Note: All Promega Technical Bulletins are available at:
www.promega.com/tbs/

II. Product Components

Product	Size	Cat.#
CellTiter-Blue™ Cell Viability Assay	100ml	G8081

Includes:

- 1 x 100ml CellTiter-Blue™ Reagent
- 1 Protocol

CellTiter-Blue™ Cell Viability Assay	10 x 100ml	G8082
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Includes:

- 10 x 100ml CellTiter-Blue™ Reagent
- 1 Protocol

Sufficient reagent is provided with each 100ml bottle for 3,936 x 20µl assays in 96-well plates or 15,744 x 5µl assays in 384-well plates when using the Beckman Coulter Biomek® 2000. When using the Beckman Coulter Biomek® FX workstation, the amount of reagent provided is sufficient for 480 x 20µl assays in 96-well plates or 1,920 x 5µl assays in 384-well plates. The single-plate Biomek® 2000 method uses a total dead volume of 480µl in the Strip Tube Holder. The single-plate Biomek® FX method includes 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.

Storage Conditions: Store the CellTiter-Blue™ Reagent frozen at -20°C protected from light. For frequent use, the reagent may be stored, tightly capped and protected from light, at 4°C or at room temperature (20–25°C) for 6–8 weeks. The product is stable for at least 10 freeze-thaw cycles. When stored and handled properly, this system is guaranteed for 12 months from the date of purchase.

III. Before You Begin

Materials to Be Supplied by the User

- fluorescent plate reader with an excitation 530–570nm and emission 580–620nm filter pair
- 96-well or 384-well opaque white or black plate suitable for cell culture

A. Preparation of Solutions

Please read the following protocol thoroughly before using the CellTiter-Blue™ Cell Viability Assay. Directions are given for performing the assay in a total volume of 120µl using 96-well plates or in a total volume of 30µl using 384-well plates with a fluorescent plate reader. However, the assay can be easily adapted to different volumes provided the 1:5 ratio of CellTiter-Blue™ Reagent volume to sample volume is preserved (e.g., 20µl CellTiter-Blue™ Reagent + 100µl of sample).

CellTiter-Blue™ Reagent Preparation

1. Equilibrate the CellTiter-Blue™ Reagent to room temperature prior to use. Protect the CellTiter-Blue™ Reagent from direct light.

Note: A 37°C water bath may be used to thaw the reagent.

B. Sample Preparation Before Automated Processing

We recommend performing the following controls when using the CellTiter-Blue™ Cell Viability Assay. For best results, empirical determination of the optimal cell number, treatment conditions and incubation time for the cell culture system may be necessary. Use identical cell numbers and volumes for the assay and the negative control samples. Wells that do not contain assay or control reactions should contain a volume of liquid, water or medium, equal to that of the assay and control wells.

No-Cell Control: Set up triplicate wells without cells to serve as the negative control to determine background fluorescence that may be present.

Untreated Cells Control: Set up triplicate wells with untreated cells to serve as a vehicle control. Add the same solvent used to deliver the test compounds to the vehicle control wells.

Optional Test Compound Control: Set up triplicate wells without cells containing the vehicle and test compound to test for possible interference with the CellTiter-Blue™ Reagent chemistry.

Positive Control for Cytotoxicity: Set up triplicate wells containing cells treated with a compound known to be toxic to the cells used in your model system.

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

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 Beckman Coulter Computer	1	609983
MP20 Eight-Tip Pipette Tool	1	609024
Gripper Tool System for Biomek® 2000	1	609001
DPC MicroMix® 5 Shaker	1	380560
DPC MicroMix® 5 Integration kit	1	380561
Pipette Tip Rack Holder	1	609121
Gray Labware Holder	2	609120
Reservoir Frame	1	372795
MicroAmp® 8-Strip Reaction Tubes	2	ABI Part# N801-0580
Strip Tube Holder	1	Promega Part# Z3341

B. Labware Requirements for the Biomek® 2000 Workstation

Description	Quantity	Ordering Information
P20 tips (rack)	1	Beckman Coulter Part# 609042
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

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

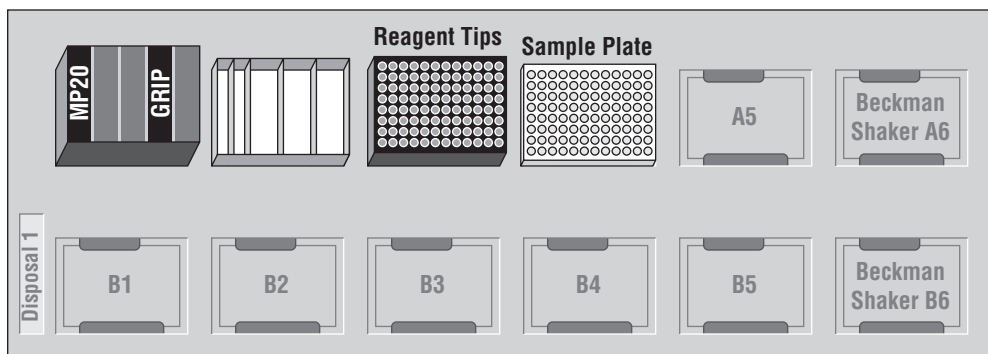


Figure 1. Deck layout for 96-well assays using the CellTiter-Blue™ Cell Viability Assay on the Biomek® 2000 workstation. This is an example of the CellTiter-Blue™ 96-well assay deck layout on the Biomek® 2000 workstation.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP20 pipetting tool; 2) Empty; 3–5) Gripper tool
A2	Reservoir frame: Strip Tube Holder containing MicroAmp® 8-Strip Reaction Tubes with 150µl of CellTiter-Blue™ Reagent per well
A3	P20 tips
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

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

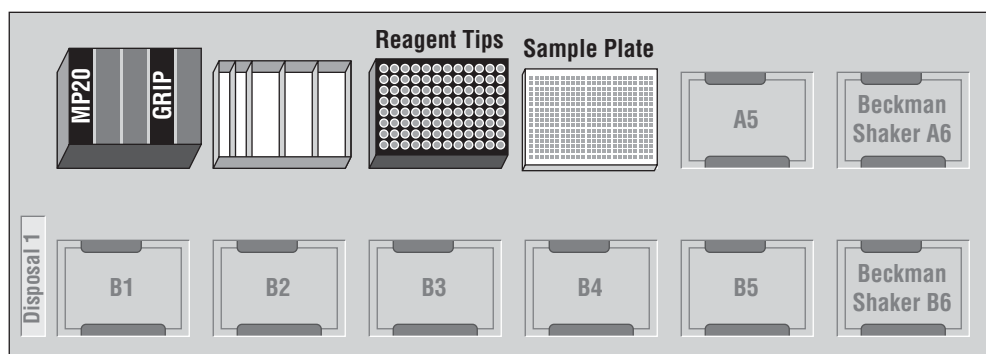


Figure 2. Deck layout for 384-well assays using the CellTiter-Blue™ Cell Viability Assay on the Biomek® 2000 workstation. This is an example of the CellTiter-Blue™ 384-well assay deck layout on the Biomek® 2000 workstation.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP20 pipetting tool; 2) Empty; 3–5) Gripper tool
A2	Reagent frame: Strip Tube Holder containing MicroAmp® 8-Strip Reaction Tubes with 150µl of CellTiter-Blue™ Reagent per well
A3	P20 tips
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

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)

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

A. Instrument Requirements for the Biomek® FX Workstation

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

Part Description	Quantity	Ordering Information
Minimum: Biomek® FX Software version 2.1		Contact Beckman Coulter
Minimum number of labware positions by 1 POD (for 96-well purification)	2	Contact Beckman Coulter
Minimum number of labware positions by 1 POD (for 384-well purification)	6	Contact Beckman Coulter
Tip Loader ALP	1	Beckman Coulter Part# 719356
Orbital Shaker ALP	1	Beckman Coulter Part# 379448
96-channel POD (for 96-well and 384-well assays)	1	Beckman Coulter Part# 719368

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 P20 tip box	1	Beckman Coulter Part# 717254
96-well, pyramid-bottom reservoir, low profile, polypropylene	1	Innovative Microplate Part# S30018
Requirements for 384-well assay using 96-well tips		
Costar® 384-well clear-bottom plate, white, polystyrene or equivalent	1	Corning Part# 3707
AP96 P20 tips (rack)	1	Beckman Coulter Part# 717254
96-well, pyramid-bottom reservoir, low profile, polypropylene	1	Innovative Microplate Part# S30018

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

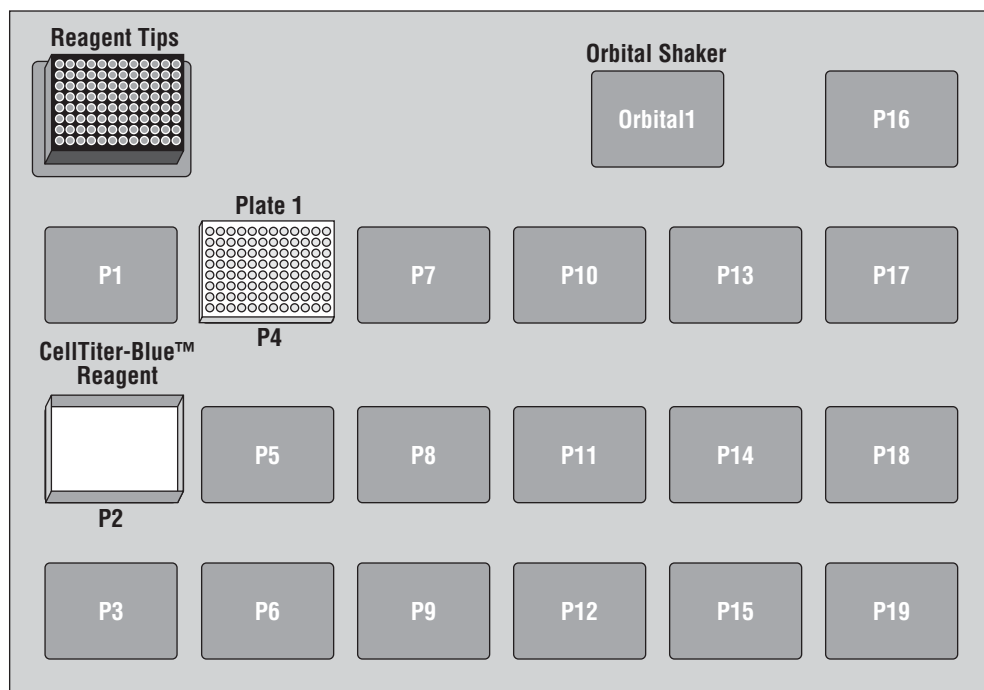


Figure 3. Deck layout for 96-well assays using the CellTiter-Blue™ Cell Viability Assay on the Biomek® FX workstation. This is an example of the CellTiter-Blue™ 96-well assay deck layout on the Biomek® FX workstation. Your specific deck layout may be different depending on your Biomek® FX configuration.

ALP Name Part Sitting on ALP

Tip loader	AP96 P20 tips
P1	Empty
P2	Pyramid-bottom reservoir, low profile, containing 17ml of CellTiter-Blue™ Reagent
P3	Empty
P4	96-well assay plate containing 100µl/well of sample, negative control or blank
P5–P19	Empty
Orbital 1	Orbital shaker ALP

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

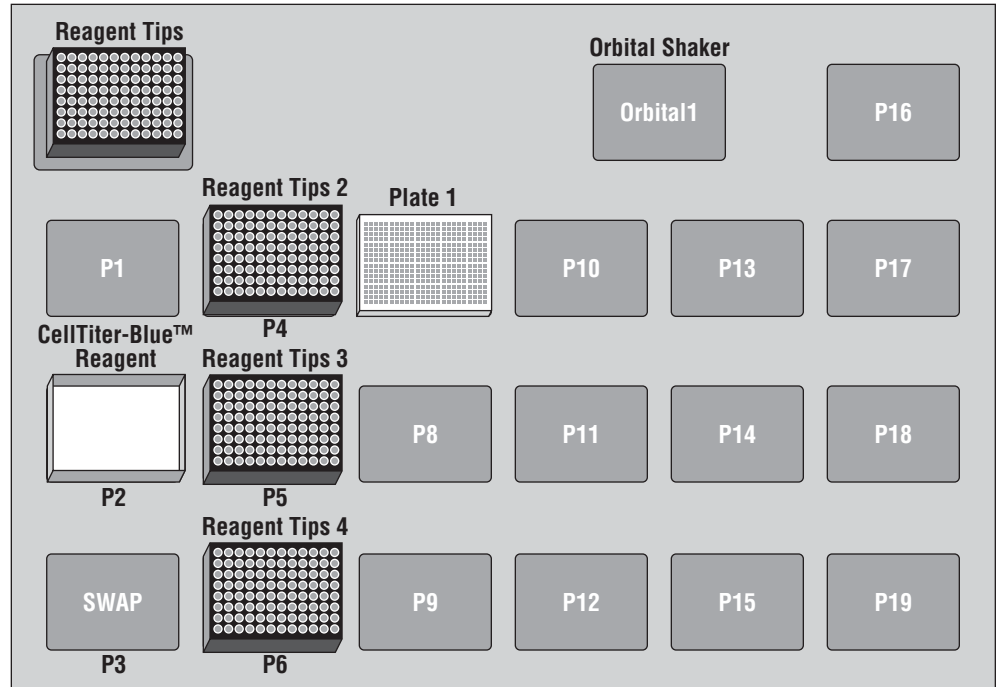


Figure 4. Deck layout for 384-well assays using a 96-channel POD and the CellTiter-Blue™ Cell Viability Assay on the Biomek® FX workstation. This is an example of the CellTiter-Blue™ Assay 384-well assay deck layout on the Biomek® FX workstation. Your specific deck layout may be different depending on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

ALP Name	Part Sitting on ALP
Tip loader	AP96 P20 tips
P1	Empty
P2	Pyramid-bottom reservoir, low profile, containing 17ml of CellTiter-Blue™ Reagent
P3	Swap space
P4	AP96 P20 tips
P5	AP96 P20 tips
P6	AP96 P20 tips
P7	384-well assay plate containing 25µl/well of sample, negative control or blank
P8–P19	Empty
Orbital 1	Orbital shaker ALP

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

The Biomek® FX automated platform 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 may be 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-Blue™ Cell Viability Assay on the Biomek® FX, it is necessary to ensure that the deck has been properly framed. Failure to do so may result in bending of tips during the method.

VI. Description of the CellTiter-Blue™ Cell Viability Assay

This overview describes general liquid handling steps required for performing 100µl or 25µl assays in a 96-well or 384-well format. The assay can be adapted to a variety of automated liquid handling robots and to different volumes, as long as the 1:5 ratio of CellTiter-Blue™ Reagent:sample volume is preserved. See Section VII for information on adaptation to liquid handling robots other than those referenced above.

A. CellTiter-Blue™ Reagent Addition

96-Well Format: The CellTiter-Blue™ Reagent (20µl) is transferred to the assay plate containing 100µl of blank, untreated control cells or treated cells in culture.

384-Well Format using 96-channel POD: The CellTiter™ Reagent (5µl) is transferred to the assay plate containing 25µl of blank, untreated control cells or treated cells in culture.

B. CellTiter-Blue™ Reagent and Sample Mixing

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 60 seconds.
3. Assay Plate Replacement. The assay plate is transferred back to its original position on the deck.

Incubate the sample plate at 37°C for a minimum of 1 hour. Manually assay samples using a fluorescent plate reader. Shake plate for 10 seconds and record fluorescence with an excitation wavelength of 560nm and an emission wavelength of 590nm.

To avoid the introduction of bubbles into the wells, the samples and reagent are not mixed with tips.



VII. General Guidelines for Adaptation to Alternative Robotic Platforms

To avoid the introduction of bubbles into the wells, do not mix the samples and reagent with tips.

Due to the concentrated nature of the CellTiter-Blue™ Reagent and the low volume of reagent delivered to each well, individual tips are used for reagent transfers. This allows for dispensing of reagent directly into each sample and enhances the accuracy of each transfer.

If each 96-or 384-well assay plate is not filled completely with samples, we recommend filling empty wells with water or media to a level that is equal to the sample volume.

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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.



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