

AUTOMATED PROTOCOL

Automated Caspase-Glo[®] Assays Protocol

Instructions for Use of Products
G8092, G8202 and G8212



Automated Caspase-Glo[®] Assays Protocol

All technical literature is available at: www.promega.com/protocols/
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 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 Caspase-Glo[®] 3/7, 8 and 9 Assays^(a,b). Specific instructions are provided for the Beckman Coulter Biomek[®] 2000 and Biomek[®] FX workstations. For information about obtaining validated methods for Caspase-Glo[®] Assays on these instruments, please visit: www.promega.com/automethods/

Please refer to the *Caspase-Glo[®] 3/7 Assay Technical Bulletin #TB323*, the *Caspase-Glo[®] 8 Assay Technical Bulletin #TB332* or the *Caspase-Glo[®] 9 Assay Technical Bulletin #TB333* to troubleshoot chemistry issues.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Caspase-Glo[®] 3/7 Assay	100ml	G8092

Includes:

- 1 × 100ml Caspase-Glo[®] 3/7 Buffer
- 1 vial Caspase-Glo[®] 3/7 Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
Caspase-Glo[®] 8 Assay	100ml	G8202

Includes:

- 1 × 100ml Caspase-Glo[®] 8 Buffer
- 1 vial Caspase-Glo[®] 8 Substrate (lyophilized)

PRODUCT	SIZE	CAT.#
Caspase-Glo[®] 9 Assay	100ml	G8212

Includes:

- 1 × 100ml Caspase-Glo[®] 9 Buffer
- 1 vial Caspase-Glo[®] 9 Substrate (lyophilized)

Sufficient reagent is provided for 768 × 100µl assays in 96-well plates or 3,072 × 25µl assays in 384-well plates when using the Beckman Coulter Biomek[®] 2000 workstation. When using the Beckman Coulter Biomek[®] FX workstation, the amount of reagent provided is sufficient for 384 × 100µl assays in 96-well plates or 1,536 × 25µl assays in 384-well plates. The single-plate Biomek[®] 2000 method uses a 2ml dead volume in the reagent trough. The single-plate Biomek[®] FX method includes a 15ml dead volume. The number of assays processed per 100ml bottle will increase if multiple plates are run and unused reagent is reused.

Storage Conditions: Store the Caspase-Glo[®] Substrates and Buffers at –20°C protected from light. The Caspase-Glo[®] Buffers may be thawed and stored at 4°C for 3 months or at room temperature for up to 4 days with no loss in signal. When stored and handled properly, these systems are guaranteed for 6 months from the date of purchase.

3. Before You Begin

Materials to Be Supplied by the User

- white-walled multiwell plates adequate for cell culture and compatible with the luminometer being used, such as Labsystems Cliniplate
- luminometer capable of reading multiwell plates

3.A. Preparation of Solutions

Please read the following protocol thoroughly before using a Caspase-Glo® Assay. Directions are given for performing the assays in a total volume of 200µl using 96-well plates or in a total volume of 50µl using 384-well plates with a plate-reading luminometer. However, the assay can be easily adapted to different volumes provided the 1:1 ratio of Caspase-Glo® Reagent volume to sample volume is preserved (e.g., 50µl of sample + 50µl Caspase-Glo® 3/7 Reagent).

Caspase-Glo® Reagent Preparation

1. Equilibrate the appropriate Caspase-Glo® Buffer and lyophilized Caspase-Glo® Substrate to room temperature prior to use.
2. Transfer the contents of the Caspase-Glo® Buffer bottle into the amber bottle containing Caspase-Glo® Substrate. Mix by swirling or inverting the contents until the substrate is thoroughly dissolved to form the Caspase-Glo® Reagent.

Storage: For specific information on storage of the reconstituted Caspase-Glo® 3/7, 8 or 9 Reagents, please refer to the Technical Bulletin for the Caspase-Glo® Assay you are using (*Caspase-Glo® 3/7 Assay Technical Bulletin #TB323*, *Caspase-Glo® 8 Assay Technical Bulletin #TB332* or *Caspase-Glo® 9 Assay Technical Bulletin #TB333*).

3.B. Sample Preparation Before Automated Processing

1. Remove 96-well or 384-well plates containing cells from the incubator, and allow plates to equilibrate to room temperature.

Notes:

1. For best results, empirical determination of the optimal cell number, apoptosis 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 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.
3. Total incubation time for the assay depends upon the culture system, but peak luminescent signal will typically be reached in 1–2 hours. For optimal results, do not exceed 3 hours incubation. In general, the luminescent signal remaining at 3 hours is greater than 50% of peak luminescence.



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 Beckman Coulter Computer	1	609983
MP200 Eight-Tip Pipette Tool	1	609025
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
Quarter Reservoir	1	372790

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

Description	Quantity	Ordering Information
P250 tips (rack)	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

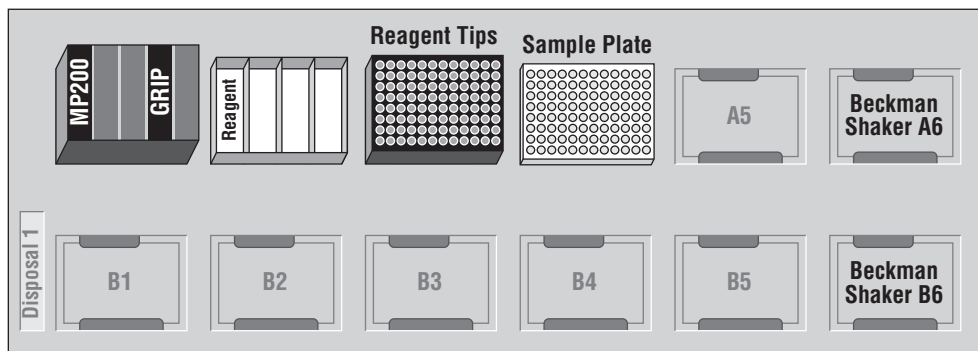


Figure 1. Deck layout for 96-well assays using the Caspase-Glo® Assay System on the Biomek® 2000 workstation. This is an example of the Caspase-Glo® 96-well assay deck layout on a Biomek® 2000 workstation. Reagent refers to the reconstituted Caspase-Glo® Reagent.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP200 pipetting tool; 2) Empty; 3–5) Gripper tool
A2	Reagent frame: quarter reservoir containing 12ml of Caspase-Glo® Reagent
A3	P250 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

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

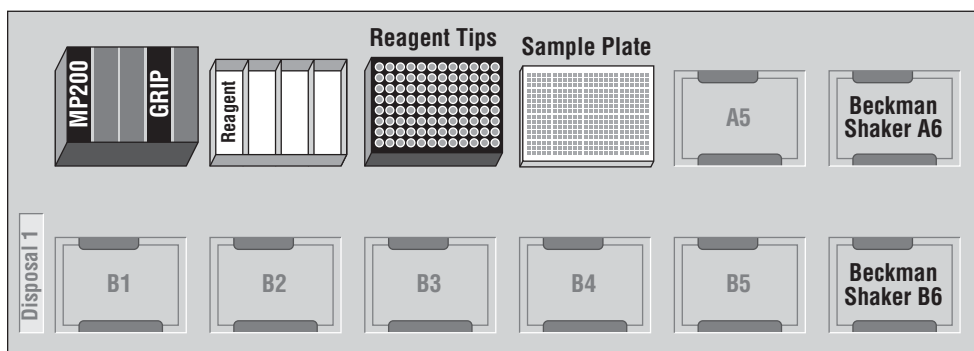


Figure 2. Deck layout for 384-well assay using a Caspase-Glo® Assay System on the Biomek® 2000 workstation. This is an example of the Caspase-Glo® 384-well assay deck layout on the Biomek® 2000 workstation. Reagent refers to the reconstituted Caspase-Glo® Reagent.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP200 pipetting tool; 2) Empty; 3–5) Gripper tool
A2	Reagent frame: quarter reservoir containing 12ml of Caspase-Glo® Reagent
A3	P250 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

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 workstation is able to run this protocol. The protocol can 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	2	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

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 tip box	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		
Costar® 384-well clear-bottom plate, white, polystyrene or equivalent	1	Corning Part# 3707
AP96 P250 tip box	1	Beckman Coulter Part# 717251
96-well, pyramid-bottom reservoir, polypropylene	1	Innovative Microplate Part# S30014

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

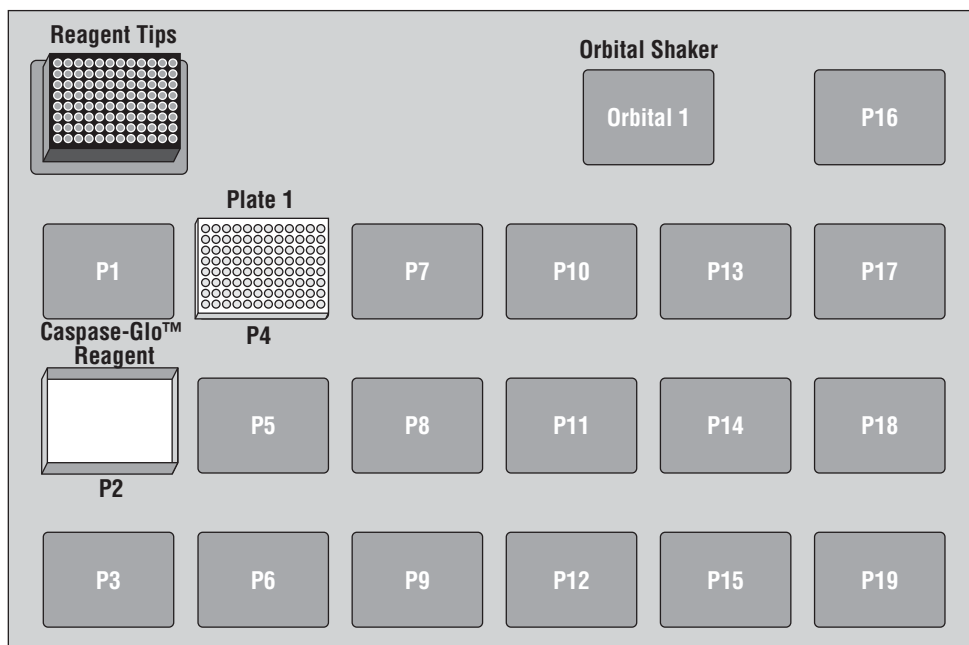


Figure 3. Deck layout for 96-well assay using the Caspase-Glo® Assay System on the Biomek® FX workstation. This is an example of the Caspase-Glo® 96-well assay deck layout on the Biomek® FX workstation. Reagent refers to the reconstituted Caspase-Glo® Reagent. Your specific deck layout may be different depending on your Biomek® FX configuration.

ALP Name	Part Sitting on ALP
Tip loader	AP96 P250 Biomek® tips
P1	Empty
P2	Pyramid-bottom reservoir containing 25ml of Caspase-Glo® 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

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

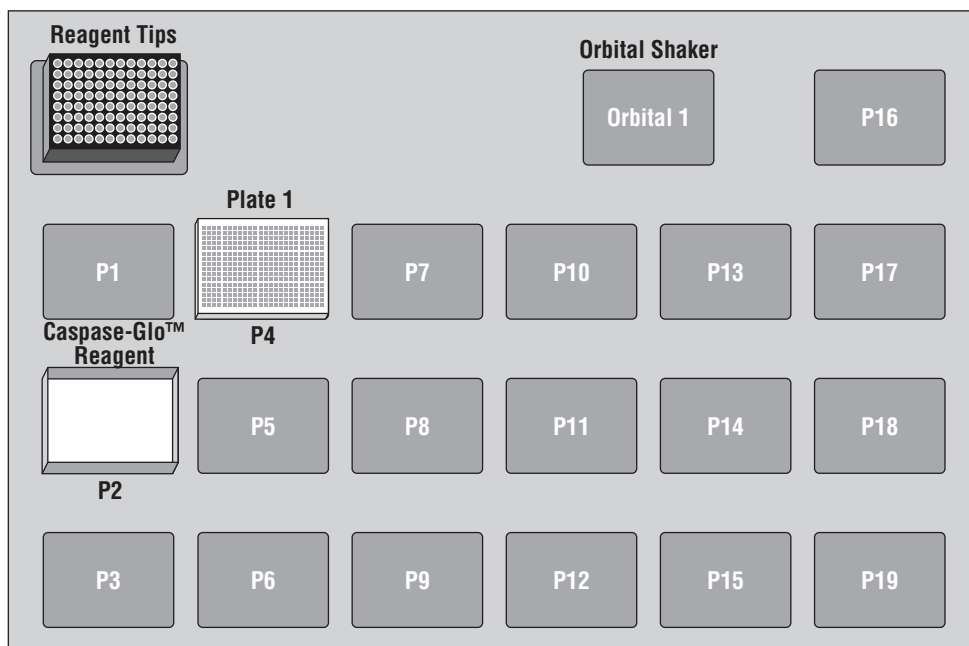


Figure 4. Deck layout for 384-well assay using a 96-channel POD and a Caspase-Glo® Assay System on the Biomek® FX workstation. This is an example of a Caspase-Glo® Assay 384-well assay deck layout on the Biomek® FX workstation. Reagent refers to the reconstituted Caspase-Glo® Reagent. Your specific deck layout may be different depending on your Biomek® FX configuration.

ALP Name	Part Sitting on ALP
Tip loader	AP96 P250 Biomek® FX tips
P1	Empty
P2	Pyramid-bottom reservoir containing 25ml of Caspase-Glo® Reagent
P3	Empty
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 automated platform allows users the flexibility to configure the robot's deck according to need. Because of this flexibility, the deck used for writing a Biomek® FX method will likely 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 any Caspase-Glo® Assay on the Biomek® FX, ensure that the deck has been properly framed. Failure to do so may result in bending of tips during the method.

6. Description of the Caspase-Glo® Assays

This overview describes general liquid-handling steps required for performing 25µl or 100µl Caspase-Glo® Assays in 384-well or 96-well format. Caspase-Glo® Assays can be adapted to a variety of automated liquid-handling robots and to different volumes, as long as the 1:1 ratio of Caspase-Glo® Reagent to sample volume is preserved. See Section 7 for information on adaptation to liquid-handling robots other than those referenced above.

6.A. Caspase-Glo® Reagent Addition

96-Well Format: The Caspase-Glo® Reagent (100µl) is transferred to the assay plate containing 100µl of culture medium control, untreated control cells, treated cells in culture or purified caspase enzyme.

384-Well Format using 96-Channel POD: The Caspase-Glo® Reagent (25µl) is transferred to the assay plate containing 25µl of culture medium control, untreated control cells, treated cells in culture or purified caspase enzyme.

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

6.B. Caspase-Glo® 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 using the shaking parameters specified in each automated method. See Table 1 for the appropriate shaking parameters.
3. **Assay Plate Replacement.** The assay plate is transferred back to its original position on the deck.

Manually read samples using a plate-reading luminometer after the predetermined incubation period.

Table 1. Shaking Parameters for the Caspase-Glo® Assays.

Automated Assay	Robotic Platform	Format	Shaking Parameters
Caspase-Glo® 3/7 Assay	Biomek® 2000 with DPC MicroMix® 5 Shaker	96-well	Form 40 Amplitude 6 1 minute
		384-well	Form 40 Amplitude 6 1 minute
	Biomek® FX with Orbital Shaker ALP	96-well	300–500rpm 1 minute
		384-well	300–500rpm 1 minute
Caspase-Glo® 8 Assay	Biomek® 2000 with DPC MicroMix® 5 Shaker	96-well	Form 40 Amplitude 6 1 minute
		384-well	Form 45 Amplitude 6 1 minute
	Biomek® FX with Orbital Shaker ALP	96-well	800rpm 1 minute
		384-well	1,000rpm 1 minute
Caspase-Glo® 9 Assay	Biomek® 2000 with DPC MicroMix® 5 Shaker	96-well	Form 40 Amplitude 6 1 minute
		384-well	Form 45 Amplitude 6 1 minute
	Biomek® FX with Orbital Shaker ALP	96-well	800rpm 1 minute
		384-well	1,000rpm 1 minute



7. 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 each well during dispensing of the reagent. No tip touches are done on the sides of the wells. This makes it possible to dispense reagent to more than one plate using a single box of tips.

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.

Due to the sensitive nature of the Caspase-Glo[®] Assays, avoid contamination with solutions containing caspase enzymes or luciferin.

8. 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. 7,148,030, 7,384,758 and 7,666,987, Japanese Pat. No. 4451663 and other patents pending.

^(b)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.

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