

# Automated Apo-ONE® Homogeneous Caspase-3/7 Assay



Automated Protocol #EP012

DESCRIPTION OF THE BECKMAN COULTER BIOMEK® 2000 and BIOMEK® FX METHODS WITH PRODUCT G7791.  
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 protocol describes automation of the Apo-ONE® Homogeneous Caspase-3/7 Assay<sup>(a)</sup>. This assay system may be used with purified enzyme preparations, cell extracts, or cultures of adherent, suspension or primary cells. Specific instructions are provided for the Beckman Coulter Biomek® 2000 and Biomek® FX Workstations. For information about obtaining the validated method, please see: [www.promega.com/automethods/](http://www.promega.com/automethods/)

Please refer to the Apo-ONE® Homogeneous Caspase-3/7 Assay Technical Bulletin #TB295 to troubleshoot chemistry issues.

**Note:** All Promega Technical Bulletins are available at: [www.promega.com/tbs/](http://www.promega.com/tbs/)



**Do not freeze** the Apo-ONE® Homogeneous Caspase-3/7 Reagent.

## II. Product Components

Product	Size	Cat.#
Apo-ONE® Homogeneous Caspase-3/7 Assay	100ml	G7791

Includes:

- 1ml Caspase Substrate Z-DEVD-R110 (100X)
- 100ml Apo-ONE® Homogeneous Caspase-3/7 Buffer
- 1 Protocol

Sufficient reagent is provided 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 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 multiplate methods are performed and leftover reagent is reused.

**Storage Conditions:** Store the Apo-ONE® Homogeneous Caspase-3/7 Assay protected from light and moisture at –20°C. Avoid multiple freeze-thaw cycles. Substrate and Buffer solutions are stable for six months from the date of purchase if stored and handled properly. Apo-ONE® Homogeneous Caspase-3/7 Reagent (Substrate diluted in Buffer) may be stored at 4°C protected from light for up to 24 hours. Do not freeze the Apo-ONE® Homogeneous Caspase-3/7 Reagent.

## III. Before You Begin

### Materials to Be Supplied by the User

- 96- or 384-well opaque white or black plate suitable for cell culture (e.g., Corning Costar products)
- Fluorescent plate reader (e.g., LabSystems Cat.# 9502887)

### A. Preparation of Solutions

Please read the following protocol carefully before using the Apo-ONE® Homogeneous Caspase-3/7 Assay on an automated workstation. Directions are given for performing the assay in a total volume of 200µl using 96-well plates, or 50µl using 384-well plates. However, the assay can be easily adapted to different volumes as long as the 1:1 ratio of Apo-ONE® Homogeneous Caspase-3/7 Reagent to sample volume is preserved.

### Apo-ONE® Homogeneous Caspase-3/7 Reagent Preparation

Thaw the 100X Substrate and Buffer and equilibrate to room temperature. Mix by inversion or vortexing. Dilute the Substrate 1:100 with Buffer to obtain the desired volume of Apo-ONE® Homogeneous Caspase-3/7 Reagent (e.g., 100µl of 100X Substrate to 9,900µl Buffer). The Apo-ONE® Homogeneous Caspase-3/7 Reagent may be stored at 4°C protected from light for up to 24 hours until use.

## B. Sample Preparation Before Automated Processing

1. Prior to starting the assay, prepare the Apo-ONE® Homogeneous Caspase-3/7 Reagent as described in Section III.A and mix thoroughly.
2. For best results, empirical determination of the optimal cell number, apoptosis induction treatment and incubation time for the cell culture system may be necessary.
3. Use identical cell numbers and volumes for assay and 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.
4. Do not mix the Apo-ONE® Homogeneous Caspase-3/7 Reagent and samples by manual pipetting. Mixing in this manner is unnecessary and may create bubbles that interfere with fluorescence readings or cross-contaminate samples. Gentle mixing may be performed using a plate shaker.
5. Total incubation time for the assay depends on the amount of caspase-3/7 present in the sample. Minimal apoptotic induction and low cell number may require an extended incubation period. The maximum recommended incubation time is 18 hours.

## IV. Automated Processing Requirements:

### Beckman Coulter Biomek® 2000 Workstation

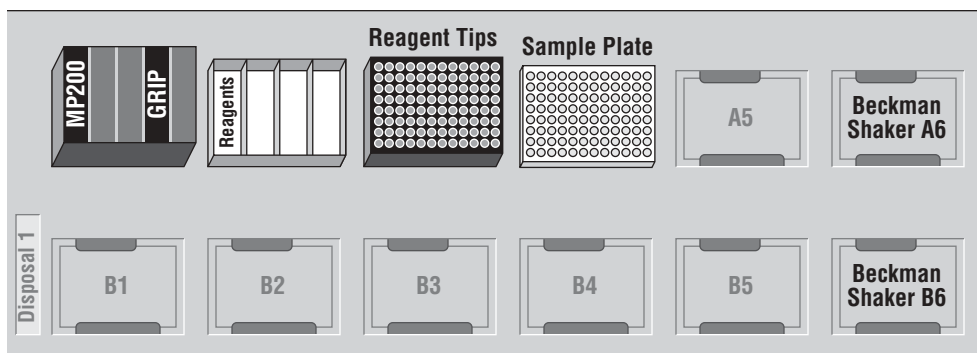
#### A. Instrument Requirements for the Biomek® 2000 Workstation

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
MP200 Pipetting Tool	1	609025
Gripper Tool System for Biomek® 2000	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
Reservoir Frame	1	372795
Quarter Reservoir	1	372790

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

Description	Quantity	Ordering Information
Biomek® 2000 P250 tips (rack)	1	Beckman Coulter Part# 372655
Costar 96-well clear-bottom plate, white, polystyrene (for 96-well assay)	1	Corning Part# 3610
Costar 384-well clear-bottom plate, white, polystyrene (for 384-well assay)	1	Corning Part# 3707

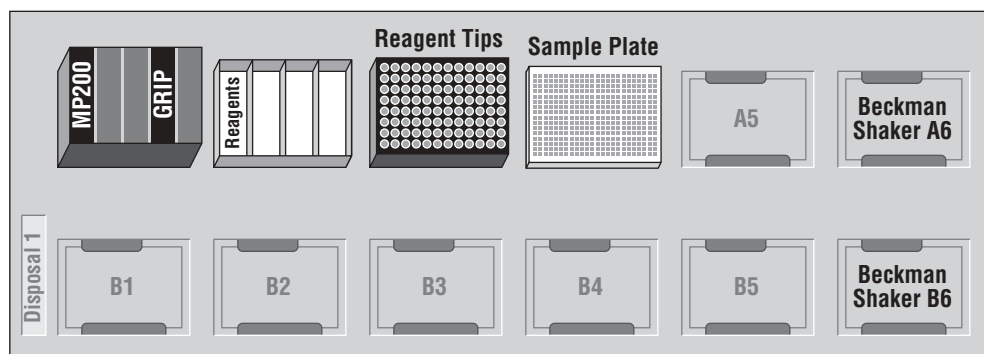
### C. Initial Deck Layout for 96-Well Assay on the Biomek® 2000 Workstation



**Figure 1. Deck layout for 96-well assay using the Apo-ONE® Homogeneous Caspase-3/7 Assay system on the Biomek® 2000 workstation.** This is an example of an Apo-ONE® Homogeneous Caspase-3/7 96-well assay deck layout on the Biomek® 2000 workstation.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP200 eight-tip pipette tool; 2) Empty; 3–5) Gripper kit
A2	Reservoir Frame: Quarter reservoir containing 12ml Apo-ONE® Homogeneous Caspase-3/7 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	Empty
B2	Empty
B3	Empty
B4	Empty
B5	Empty
B6	Beckman Coulter shaker integration plate holder

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



**Figure 2. Deck layout for 384-well assay using the Apo-ONE® Homogeneous Caspase 3/7 Assay system on the Biomek® 2000 workstation.** This is an example of an Apo-ONE® Homogeneous Caspase-3/7 384-well assay deck layout on the Biomek® 2000 workstation.

Position Name	Part Sitting on Deck Position
A1	Tool rack: 1) MP200 eight-tip pipette tool; 2) Empty; 3–5) Gripper kit
A2	Reagent Frame: Quarter reservoir containing 12ml Apo-ONE® Homogeneous Caspase-3/7 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	Empty
B2	Empty
B3	Empty
B4	Empty
B5	Empty
B6	Beckman Coulter shaker integration plate holder

#### E. Pre-Run Biomek® 2000-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/default.asp](http://www.promega.com/automethods/beckman/biomek2000/default.asp))

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

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

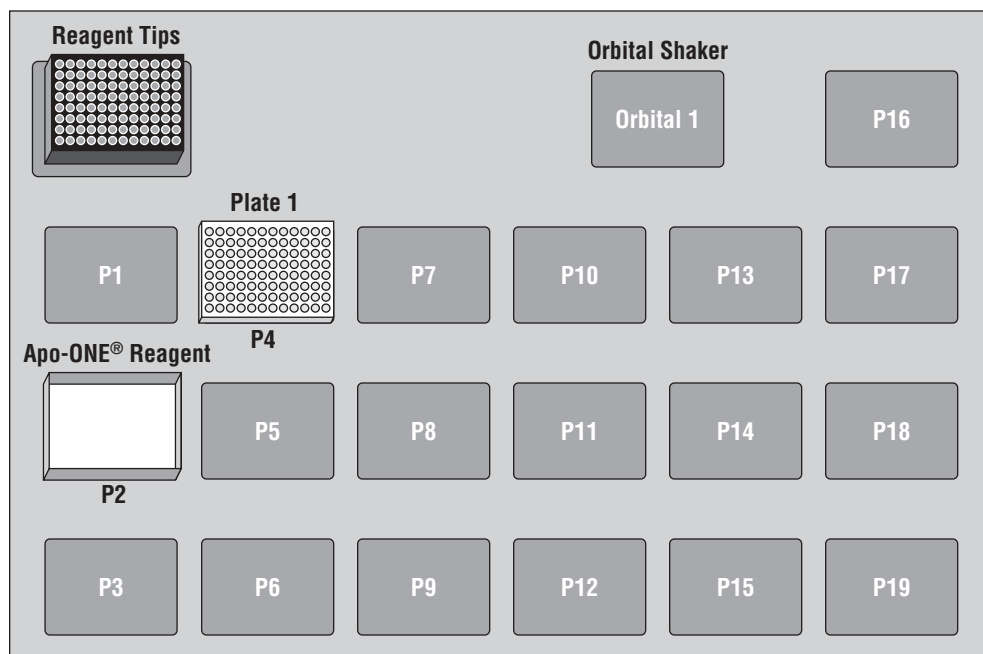
Any single-arm multichannel Biomek® FX is able to run this protocol. The protocol can also be adapted for a dual-arm Biomek® FX with at least one multichannel pod.

<b>Part Description</b>	<b>Quantity</b>	<b>Ordering Information</b>
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

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

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

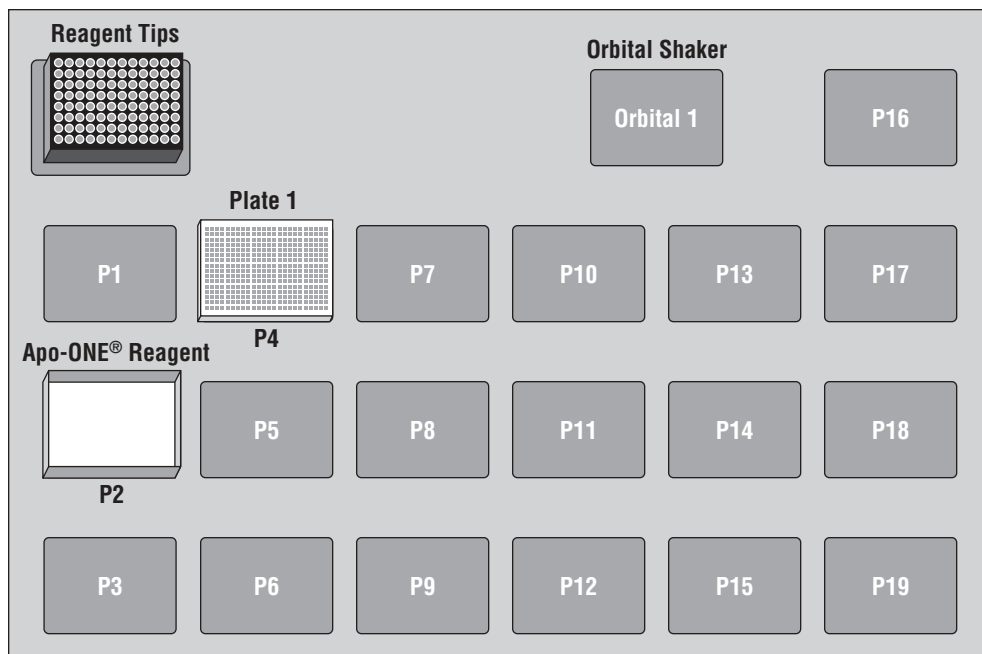
### C. Initial Deck Layout for 96-Well Assay on the Biomek® FX Workstation



**Figure 3. Deck layout for 96-well assay using the Apo-ONE® Homogeneous Caspase 3/7 Assay system on the Biomek® FX workstation.** This is an example of an Apo-ONE® Homogeneous Caspase-3/7 96-well assay deck layout on the Biomek® FX. 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 Apo-ONE® Homogeneous Caspase-3/7 Reagent
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 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 Apo-ONE® Homogeneous Caspase-3/7 Assay system on the Biomek® FX workstation.** This is an example of an Apo-ONE® Homogeneous Caspase-3/7 384-well assay deck layout on the Biomek® FX. 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 Apo-ONE® Homogeneous Caspase-3/7 Reagent
P4	384-well assay plate containing 25µl/well of sample, negative control or blank
P5–P19	Empty
Orbital 1	Orbital shaker ALP

#### 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 \(www.promega.com/automethods/beckman/biomekfx/default.asp\)](http://www.promega.com/automethods/beckman/biomekfx/default.asp).

Prior to the first run of the Apo-ONE® Homogeneous Caspase-3/7 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 Apo-ONE® Homogeneous Caspase-3/7 Assay

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 Apo-ONE® Homogeneous Caspase-3/7 Reagent:sample volume is preserved. See Section VII for information on adaptation to liquid handling robots other than those referenced above.

### A. Apo-ONE® Homogeneous Caspase-3/7 Reagent Addition

**96-Well Format:** Homogeneous Caspase-3/7 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 POD:** Homogeneous Caspase-3/7 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.

### B. Apo-ONE® Homogeneous Caspase-3/7 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 60 seconds.
3. Assay Plate Replacement. The assay plate is transferred back to its original position on the deck.

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

## VII. 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 during dispensing of 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.



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