Miniaturized Cell-Based and Biochemical Assays Using the Deerac Fluidics Equator™ NS-808 Eight-Tip Pipetting System

Automated Protocol #EP027

DESCRIPTION OF THE DEERAC FLUIDICS™ EQUATOR™ METHOD FOR PRODUCTS E4910, E4920, V6712, V6713, G7571, G7572, G7573, G8091, G8092, V8752, V8762, V8772, V8782, V8792, V8802 AND V8812.
All technical literature is available on the Internet at www.promega.com
Please visit the web site to verify that you are using the most current version of this Automated Protocol.

I. Description

This document describes automation and miniaturization of the chemistries listed below. Specific instructions are provided for the Deerac Fluidics Equator™ NS-808 Eight-Tip Pipetting System. Low-volume 384- and 1,536-well methods may be requested at: **www.promega.com/automethods/**

- CellTiter-Glo® Luminescent Cell Viability Assay, an assay based on the quantitation of ATP in metabolically active cells.
- Caspase-Glo® 3/7 Assay, a luminescent method to detect caspase 3/7 activity.
- Chroma-Glo™ Luciferase Assay System, a dual-color, normalized assay for luciferase reporter gene expression.

- P450-Glo™ CYP450 Assays, a homogeneous, luminescent method for measuring cytochrome P450 activity.
- Kinase-Glo® Luminescent Kinase Assay, a homogeneous method to determine purified kinase activity by quantifying the amount of ATP remaining following a kinase reaction.

II. Reagent Systems Technical Information

Detailed descriptions of the assay chemistries and reagents, as well as trouble-shooting information are provided in the technical literature supplied with each product. Custom reagent volumes are also available. The technical literature specific for each system is listed below. All Promega technical literature is available on our Web site at: **www.promega.com/tbs/**

- *CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin* #TB288.
- *Caspase-Glo® 3/7 Assay Technical Bulletin* #TB323.
- *Chroma-Glo™ Luciferase Assay System Technical Manual* #TM062.
- *P450-Glo™ CYP450 Assays Technical Bulletin* #TB325.
- *Kinase-Glo® Luminescent Kinase Assay Technical Bulletin* #TB318.

III. Before You Begin

A. Materials to Be Supplied by the User

Solution compositions are provided in Section VII.

All Assays

- Low-Volume 384-well (Corning Cat.# 3673) or 1,536-well (Corning Cat.# 3937) opaque white plates suitable for cell culture or biochemical assays
- Luminometer or CCD camera imaging device capable of reading multiwell plates in high density formats

P450-Glo™ Assays

- 1M potassium phosphate buffer (pH 7.4)
- Distilled, deionized, or nuclease-free water
- Active cytochrome P450 preparation that includes cytochrome P450 reductase (See the *P450-Glo™ CYP450 Assays Technical Bulletin* #TB325 for supplier information)
- Preparation that lacks cytochrome P450 activity for negative control reactions
- 2X NADPH regenerating system
- Luminescence plate reader (e.g., BMG FLUOstar OPTIMA or equivalent) capable of reading multiwell plates
- 96-Well U-Bottom Collection Plate (Promega Cat.# A9161)

Kinase-Glo® Assays

- ATP
- Kinase substrate
- Kinase reaction buffer
- Kinase of interest
- Distilled, Deionized, or Nuclease Free Water
- 96-Well, U-Bottom Collection Plate (Promega Cat.# A9161)

B. Preparation of Buffers and Solutions

Please read the following protocol carefully before beginning an assay. Directions are given for performing the assays in either 384-well or 1,536-well formats. However, each assay can be easily adapted to different volumes providing that the ratio of assay components is kept constant across the range of volumes tested.

1. Before beginning each assay, prepare each reagent according to the instructions listed in the technical literature supplied with the product.
2. Equilibrate each reagent to room temperature, where appropriate, before beginning the assay.

C. Sample Preparation Before Automated Processing

For best results using Promega cell-based assay chemistries, empirical determination of the optimal cell number, induction treatment, and incubation time for the cell culture system may be necessary. For cell-based assays, equilibrate the sample plate and its contents to room temperature for approximately 30 minutes prior to performing the assay. See the Technical Bulletin supplied with each system for specific recommendations.

Use identical cell numbers and volumes for test and negative control samples.

IV. Automated Processing Requirements

A. Instrument Requirements

The following is a list of parts and their corresponding part numbers required for use of Promega cell-based and biochemical assays on the Deerac Fluidics™ Equator™ NS-808 Eight-Tip Pipetting System.

Part Description	Quantity	Deerac Fluidics™ Part Number
Equator™ NS-808 Eight-Tip Pipetting System	1	ALL002-01
Active Wash Station, (includes peristaltic pump and tubing)	1	ALL019-01
Reservoir (Standard, provided with Equator™ System)	1	ALL024-02

B. Initial Deck Layout for Low-Volume 384- and 1,536-Well Cell-Based Assays

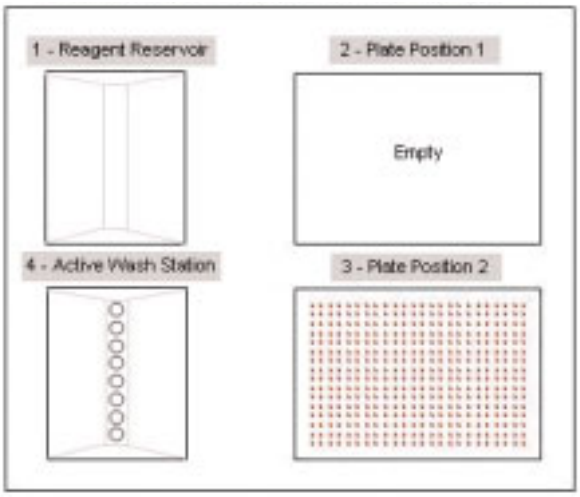

Figure 1. Initial deck configuration for low-volume, 384-well cell-based assays.

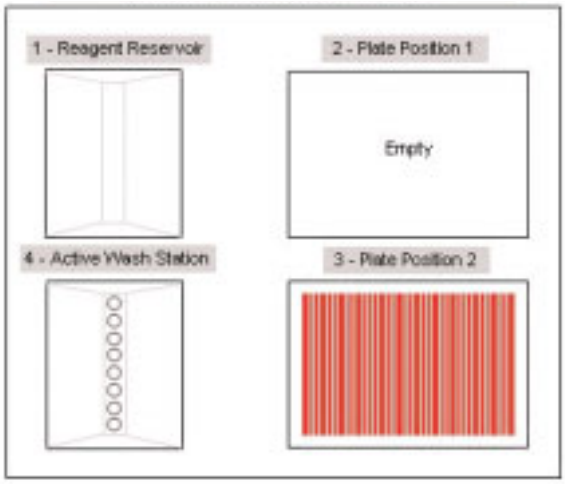

Figure 2. Initial deck configuration for low-volume, 1,536-well cell-based assays.

Position	Equipment at Deck Position
1	Reagent Reservoir containing either appropriate media with cells, or reconstituted, room temperature reagent
2	Plate Position 1: Empty
3	Plate Position 2: Low-volume 384- or 1,536-well plate (Use tissue culture-treated plate if using adherent cell lines)
4	Active Wash Station

C. Initial Deck Layout for Low-Volume 384- and 1,536-Well Biochemical Assays

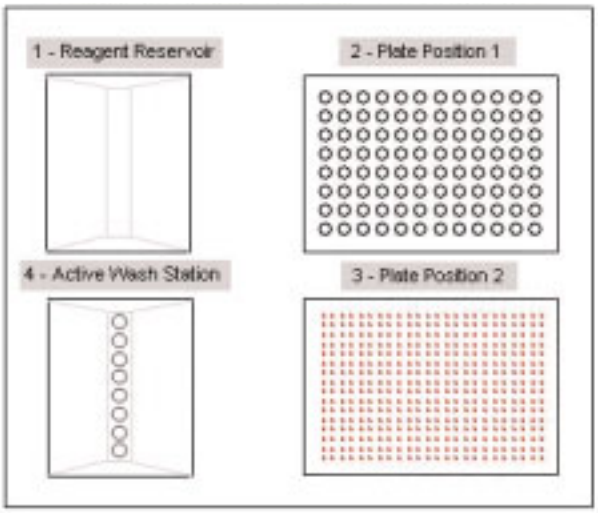

Figure 3. Initial deck configuration for low-volume, 384-well biochemical assays.

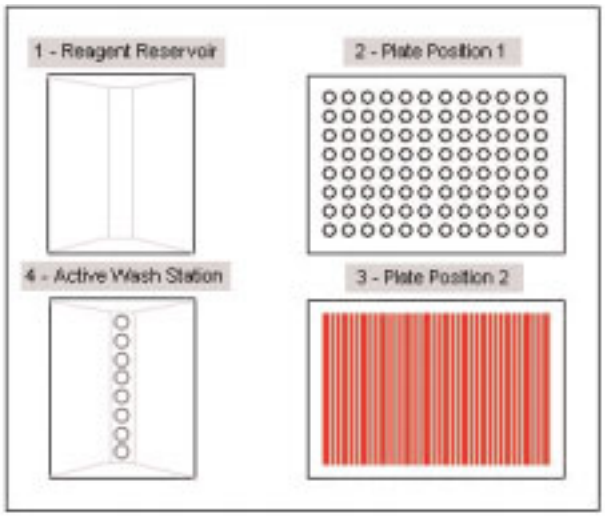

Figure 4. Initial deck configuration for low-volume, 1,536-well biochemical assays.

Position	Equipment at Deck Position
1	Reagent Reservoir containing appropriate reconstituted, room temperature reagent
2	Plate Position 1: 96-well, U-bottom plate containing remaining assay components (for P450-Glo™ Assays, Column 1 contains Cytochrome P450 Reaction Mixture and Column 3 contains NADPH Regenerating System; for Kinase-Glo® Assays, Column 1 contains Enzyme/Substrate combinations and Column 3 contains ATP)
3	Plate Position 2: Low-volume 384- or 1,536-well plate
4	Active Wash Station

D. Equator™ NS-808 Eight-Tip Pipetting System Specific Pre-Run Recommendations

To run spot station™ methods on the Deerac Fluidics™ Equator™ NS 808, the correct Liquid Classes, Plate Files, and Maximum Needle Volume must be included in the system configuration.

Liquid Classes

In spot station™, the software used to run methods on the Equator™ NS 808, a liquid class is a set of dispenser parameters used to define the properties of a liquid. Selection of the correct liquid class is important to ensure dispensing accuracy. The liquid class used in Promega methods is based on the Water liquid class. The Pump Pressure has been decreased from 1,400 to 900 to avoid splashing in high density plates. This new liquid class is called “low pressure dispense”. To use the methods presented here, a liquid class of the same name and containing the same parameters must be created. This liquid class must be loaded into the spot station™ configuration through the **Configure-Liquids** menu. When “Configure Liquids” is selected, the **Liquids** dialog will be displayed. Creation of a new liquid class is accomplished as follows:

1. Select the predefined liquid class **Water**.
2. **Copy** this liquid class and then enter the name of the new class “low pressure dispense”.
3. Change the Pump Pressure to 900.
4. **Save**.

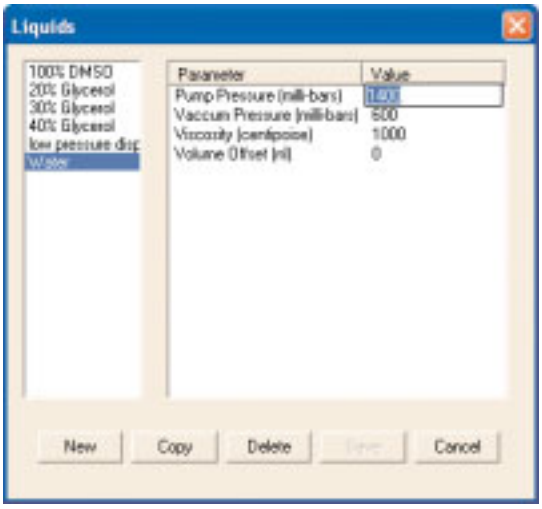

Figure 5. Editing liquid class parameters.

Plate Files

Custom plates that have been created with the Deerac Fluidics™ Plate Designer software must be imported into the spot station™ configuration prior to use. Promega has created plate files that can be imported and used with the associated methods. Additional plates can be designed and the methods rewritten. The **Import Custom Plate** utility is available through the **File** menu. When "Import Custom Plate" is selected, you will be prompted to select a custom plate file. Promega plate files are stored in the Promega Plates folder. Custom plates are stored in XML format and have a .XML file extension. When you have selected a plate file, select **Open** to import that file. A message will be displayed, indicating whether the import has been successful. The custom plate may now be selected for use. Note that spot station™ will display a message if the wells are not on a 9mm pitch. Click "**Yes**" to proceed.

Global Maximum Needle Volume

To run the methods described here on the Equator™ System, the Global Maximum Needle Volume must be set at 120,000nl. This can be altered under the **Tools** menu, by selecting "Options" then "Aspirate".

Active Wash Station

Ensure that the clean rinse container is filled with the appropriate rinse solution, and the waste container is emptied before beginning each run.

Use of Other Plates

Each method for the Equator™ NS 808 is set up to use the plates specifically described in the software. The plates described in Section III.A should be used when running the methods described in this protocol. If other plates are used, the plate definitions will need to be created using the Plate Designer software. The existing method should then be used as a template to create a new method.

V. Description of Low-Volume 384- and 1,536-Well Assays

This overview describes the general liquid handling steps required to perform low-volume 384-, and 1,536-well assays. The assays can be adapted to different volumes provided that the 1:1 ratio of reagent volume to cell or reaction volume is preserved.

A. CellTiter-Glo®, Caspase-Glo® 3/7 and Chroma-Glo™ Assays

1. Mammalian cells in culture medium and reagents are dispensed as indicated in Table 1. The time at which the cells are dispensed prior to the assay will vary depending on the type of cells and the nature of the assay being performed.

Table 1. Cell Culture and Reagent Volumes for 384- and 1,536-Well Assays Using the CellTiter-Glo®, Caspase-Glo® 3/7 and Chroma-Glo™ Assay Systems.

Step	LV384-Well Format	1,536-Well Format
Cell Addition		
Dispense mammalian cells in culture medium	4.0µl	2.0µl
Reagent Addition		
Dispense reagent to assay plate	5.0µl	2.5µl

2. Allow the assay plate to incubate at room temperature to stabilize the luminescent signal. Incubation times are as follows: CellTiter-Glo® Assay, 10 Minutes; Caspase-Glo™ 3/7 Assay, 30 Minutes; Chroma-Glo™Assay, 5 Minutes.
3. Measure the luminescence of each sample using a plate-reading luminometer or CCD Imager.

   Note: Filter sets are required to read the red and green luminescence generated using the Chroma-Glo™ Assay. We recommend using a 510/60nm and 610 long-pass filter with the luminometer or CCD Imager used to read Chroma-Glo™ Assay plates. For maximal sensitivity, measure luminescence with the red filter before measuring luminescence with the green filter. For additional information, see the *Chroma-Glo™ Luciferase Assay System Technical Manual* #TM062.

B. P450-Glo™ Assays

Prior to beginning the method, dispense vehicle controls or test compounds into the assay plate. The volume of test compound added to each well of the assay plate is 750nl for the 6µl LV384-well method, and 375nl for the 3µl 1,536-well method.

Table 2. Reagent Volumes for 384- and 1,536-Well Assays Using the P450-Glo™ Assay System.

Reagent	LV384-Well Format (6µl)	1,536-Well Format (3µl)
4X Cytochrome P450 Reaction Mixture	750nl	375nl
2X NADPH Regenerating System	1,500nl	750nl
Luciferin Detection Reagent	3,000nl	1,500nl

1. Add Cytochrome P450 Reaction Mixture (Table 2).
2. Incubate for 10 minutes at room temperature.
3. Add NADPH Regenerating System (Table 2).
4. Incubate for 60 minutes.
5. Add Luciferin Detection Reagent (Table 2).
6. Incubate for 20 minutes at room temperature.
7. Measure the luminescence of each sample using a plate reading luminometer or CCD-based imager.

C. Kinase-Glo™ Assays

Prior to beginning the method, dispense vehicle controls or test compounds into the assay plate. The volume of test compound added to each well of the assay plate is 500nl for a 10µl LV384-well method, and 250nl for a 5µl 1,536-well method. Equilibrate the sample plate and its contents to room temperature for approximately 30 minutes before performing the assay.

Table 3. Reagent Volumes for 384- and 1,536-Well Assays Using the Kinase-Glo® Assay System.

Reagent	LV384-Well Format (10µl)	1,536-Well Format (5µl)
Kinase/Substrate Mixture	2.0µl	1.0µll
ATP	2.5µl	1.25µl
Kinase-Glo® Reagent	5.0µl	2.5µl

1. Dispense Kinase/Substrate Mixture (Table 3).
2. Dispense ATP (Table 3).
3. Incubate at room temperature for 20 minutes.
4. Add Kinase-Glo® Reagent (Table 3).
5. Incubate at room temperature for 20 minutes.
6. Measure the luminescence of each sample using a plate-reading luminometer or CCD-based imager.

VI. General Guidelines for Adaptation to the Equator™ NS-808 Eight Tip Pipetting System

The liquid classes provided for use with this automated method were specifically determined for the reagents supplied with the CellTiter-Glo®, Caspase-Glo®, Chroma-Glo™, Kinase-Glo® and P450-Glo™ assays. If other reagents are used, liquid classes will need to be determined accordingly.

The methods described in this automated protocol use the Active Wash Station. If either the Passive Wash or Fast Wash options are used, methods should be altered accordingly.

VII. Composition of Buffers and Solutions

1M potassium phosphate buffer (pH 7.4)

13.94g	potassium phosphate, dibasic, anhydrous (K_2HPO_4)
2.72g	potassium phosphate, monobasic, anhydrous (KH_2PO_4)

Bring the volume to approximately 90ml with deionized water. Adjust to pH 7.4 with KOH or H_3PO_4. Add deionized water to a final volume of 100ml. Use to prepare 2X cytochrome P450/KPO_4/substrate reaction mixtures.

2X NADPH regenerating system

2.6mM	NADP+
6.6mM	glucose-6-phosphate
0.4u/ml	glucose-6-phosphate dehydrogenase
6.6mM	$MgCl_2$

To limit activity, keep the glucose-6-phosphate dehydrogenase separate from the other reaction components until ready to use. Concentrated stocks (e.g., 20X or 100X) can be prepared in advance. Store at –20°C.

2X NADPH regenerating system for use with CYP3A4

2.6mM	NADP+
6.6mM	glucose-6-phosphate
0.4u/ml	glucose-6-phosphate dehydrogenase
6.6mM	$MgCl_2$
400mM	potassium phosphate buffer (pH 7.4)

To limit activity, keep the glucose-6-phosphate dehydrogenase separate from the other reaction components until ready to use. Concentrated stocks (e.g., 20X or 100X) can be prepared in advance. Store at –20°C.

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