User Manual

PathHunter® Flash Detection Kit

For Use with PathHunter Cell Lines or Cell Lines Generated with ProLabel®/ProLink™ Vectors

Catalog Number: 93-0247 Series

Document Number 70-257 Revision 13

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Important: Please read this entire user manual before proceeding with the assay.

For additional information or Technical Support, see contact information at the bottom of this page.

Overview

The PathHunter Flash Detection Kit is to be used with PathHunter Cell Lines or cell lines made with ProLabel®/ProLink™ expression or cloning vectors. This easy-to-use kit has successfully been run in both 96-well and 384-well microplate format. The resulting chemiluminescent signal can be read with any standard plate reader. To determine instrument compatibility, visit discoverx.com/instrument-compatibility

PathHunter products utilize the Enzyme Fragment Complementation (EFC) technology for studying protein-protein interactions, protein translocation, receptor trafficking, and receptor internalization, all of which involve the complementation of two β-galactosidase (β-gal) fragments. The larger β-gal fragment is called Enzyme Acceptor (EA) and the smaller fragment is termed Enzyme Donor (ED; also known as ProLink or ProLabel). The two fragments are inactive when apart. However, when they complement, they form a functional enzyme that hydrolyzes the substrate to generate a chemiluminescent signal. The detection reagents do not contain either the EA or ED fragments of β-gal, and therefore must be used in conjunction with cells that express both components in order to obtain a detectable signal.

Materials Provided

Catalog Number	**93-0247M**	**93-0247**	**93-0247L**	**93-0247XL**
Number of Plates per Kit	**2**	**10**	**100**	**400**
Data Points per 96-Well Plate	~200	~1000	~N/A	~N/A
Data Points per 384-Well Plate	~800	~4000	~40,000	~160,000
List of Components	**Volume per Bottle (mL)**			
Flash Cell Assay Buffer	5	25	200	800
Flash Substrate	20	100	800	3200
Total Volume	**25**	**125**	**1000**	**4000**

Storage Conditions

Upon receipt, store reagents at -20°C. Thaw reagents at room temperature before use. Thawed reagents are stable for 4 days when stored at 2-8°C. The reagents can tolerate up to three freeze-thaw cycles with no impact on performance.

Additional Materials Required

The following equipment and additional materials are required to perform these assays:

Material	Ordering Information
96-Well Green, V-Bottom, Untreated, Non-Sterile Dilution Plates	92-0011
Multimode or luminescence plate reader	Refer to the Instrument Compatibility Chart at discoverx.com/instrument-compatibility
Sterile disposable reagent reservoir	ThermoFisher Scientific, Cat. No. 8094 or similar
Single and multichannel micropipettes and pipette tips (10 µL-1000 µL)	
Polypropylene tubes (50 mL and 15 mL)	
Microcentrifuge tubes (1.5 mL)	
Tissue culture disposable pipettes (1 mL-25 mL) and tissue culture flasks (T25 and T75 flasks, etc.)	

Assay Detection Protocol

The following procedure details the assay detection protocol to be used with PathHunter cell lines. Please refer to your cell line user manual for specific cell line and assay preparation details. Detection reagents must be prepared as a working solution prior to use. Once prepared, the working solution is stable for up to 24 hours at room temperature with no impact on assay performance.

Working Detection Solution for 96- or 384-Well Formats		
Components	Volume Ratio	Volume per Plate (mL)
Flash Cell Assay Buffer	1	2.5
Flash Substrate	4	10
Total Volume		**12.5**

1. Prepare a stock of the Flash Working Detection Solution in a 15 mL polypropylene tube or reagent reservoir by mixing 1 part of Flash Cell Assay Buffer and 4 parts of Flash Substrate.

   The stock solution should be used with 24 hours of preparation.

2. Addition of the Working Detection Solution to the assay plate (equivalent to 100% of the assay volume):

   2.1. For a 96-well format, add 110 µL of the Working Detection Solution
   2.2. For a 384-well , add 25 µL of the Working Detection Solution

   Do not pipet up and down or vortex the plates to mix the detection reagents with the cells.

3. Incubate the assay plate for 1 hour at room temperature in the dark.

   Working Detection Solution is light sensitive, thus incubation in the dark is necessary.

4. Read samples on a standard luminescence plate reader at 0.1 to 1 second/well for photomultiplier tube readers, or 5-10 seconds for imager. The actual signal characteristics over time are affected by lab conditions such as temperature. The user should establish an optimal read time accordingly. Luminesce readout collects signals from al wavelengths. Some instrument manufacturers may include a cutoff filter at high wavelengths, but usually no wavelength setting is needed for luminesce readout.

   After addition of reagents, the samples should be read within 2 hours.

Data analysis can be performed using any standard statistical software (e.g GraphPad Prism, Molecular Devices Softmax Pro, Biotek Instruments Gen5, Microsoft Excel, etc.).

For questions on using this product, please contact Technical Support at 1.866.448.4864 or DRX_SupportUS@eurofinsUS.com

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