User Manual

PathHunter® Detection Kit

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

For Assay Kits:

93-0001M:2-Plate Kit
93-0001:10-Plate Kit
93-0001L:100-Plate Kit
93-0001XL:500-Plate Kit
93-0001-HTS400:1000-Plate Kit
93-0001-HTS800:2000-Plate Kit
93-0001-HTS1M: 2500-Plate Kit

Document Number 70-142 Revision 9

Please read the entire User Manual before proceeding with the assay.

1. Overview

The PathHunter Detection Kit is to be used with PathHunter Cell Lines or cell lines made with ProLabel®/ProLink™ expression or cloning vectors. The easy-to-use and has been successfully run in both 96-well and 384-well microplate formats. The resulting chemiluminescent signal can be read with any standard plate reader.

PathHunter products utilize the Enzyme Fragment Complementation (EFC) technology for studying many modes of actions (MOAs), including reporter signaling, protein- protein interactions, receptor- dimerization, 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) while the smaller fragment is termed Enzyme Donor (ED; also known as ProLabel or ProLink). The two fragments are inactive when apart but the two parts complement with one another to form a functional enzyme that hydrolyzes the substrate included in the PathHunter Detection kit to generate a chemiluminescent signal that is reflective of the level of active enzyme present. The detection reagents do not contain either the EA or ED enzyme fragments of β-gal, and therefore must be used in conjunction with cells that express both components such as PathHunter cell lines or cell lines developed with ProLabel/ProLink vectors in order to obtain a detectable signal.

2. Materials Provided

Catalog Number	**93-0001M**	**93-0001**	**93-0001L**	**93-0001XL**	**93-0001-HTS400**	**93-0001-HTS800**	**93-0001-HTS1M**
Number of Plates*	2	10	100	500	1000	2000	2500
96-well, No. of data points	~200	~1000	n/a	n/a	n/a	n/a	n/a
384-well, No. of data points	~800	~4,000	~40,000	~200,000	~400,000	~800,000	~1000,000
Kit Components	**Total Volume (mL)**						
Cell Assay Buffer	11.4	57	418	1825	3650	7300	9125
Substrate Reagent 1	3	15	110	480	960	1920	2400
Substrate Reagent 2	0.6	3	22	96	192	384	480
Total Kit Volume	15	75	550	2401	4802	9604	12005

***Note**- Plates not provided with the kit.

3. Storage Conditions

Store reagents at -20°C upon arrival. It is important to thaw the kit from -20°C to room temperature at least 24 hours prior to using the kit. After thawing the kit to room temperature, leave it at 2-8°C overnight before use. Ensure that the reagents are at room temperature for best performance. The detection kit is stable until the expiration date indicated on the kit box outer label if left frozen at -20°C. After thawing, store reagents for up to 1 month at 2-8°C.

For long-term storage, aliquots of all the components may be re-frozen in opaque containers at -20°C. The reagents can be thawed and frozen for a total of 3 times without loss in performance.

4. Additional Materials Needed

Required Materials
PathHunter cell line expressing EA and ED
15 mL Polypropylene tube (or larger, if necessary)
Multimode or luminescence plate reader
Disposable reagent reservoir (Thermo Scientific, Cat. No. 8094 or similar)

5. 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 at room temperature for up to 24 hours with no impact on assay performance.

Working Detection Solution for 96 or 384-well Format		
Components	**Volume Ratio**	**Volume per Plate (mL)**
Cell Assay Buffer	19	5.7
Substrate Reagent 1	5	1.5
Substrate Reagent 2	1	0.3
Total Volume		7.5

1. Prepare a stock of Working Detection Solution in a 15 mL polypropylene tube or reagent reservoir by mixing 19 parts of cell assay buffer, 5 parts of Substrate Reagent, and 1 part of Substrate Reagent 2.

Make stock within 24 hours of use.

2. Add 55 µL (for 96-well format) or 12.5 µL (for 384-well format) [equivalent to 50% of the assay volume] of Working Detection Solution to all wells of the assay plate.

Do not pipette up and down in the vial to mix or vortex plates.

3. Incubate 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. Luminescence readout collects signal from all wavelengths. Some instrument manufacturers may include a cutoff filter at high wavelengths, but usually no wavelength setting is needed for luminescence readout.

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

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

6. Supplemental Information

Instrument Compatibility Chart	
Compatible with any luminometer. Select examples indicated below.	
Berthold Technologies: Mithras LB940, CentroLIApc	**Molecular Devices:** FLIPR, SpectraMax M3/ M4/M5/M5e, FlexStation 3, SpectraMax L
Biotek: Synergy 2	**Perkin Elmer:** TopCount, Victor 2 or V, Fusion, LumiCount, Envision, Micro-beta (Trilux), Viewlux, Northstar, EnSpire
BMG: PheraStar, Cytostar, LumiStar	**Promega:** GloMax systems
Caliper: LabChip 3000 & EZ Reader	**Tecan:** Ultra, Evolution
GE: LEAD seeker, Farcyte	**Thermo Scientific:** Luminoskan Ascent
Hamamatsu: FDS6000, FDSS/RayCatcher	**Turner BioSystems:** Modulus Microplate

Limited Use License Agreement

These products may be covered by issued US and/or foreign patents and patent applications and are subject to Limited Use Label License.

Please visit www.discoverx.com/license for limited use label license and trademark information for this product.

Contact Information

Eurofins DiscoverX

42501 Albrae Street, Fremont, CA 94538

Web: discoverx.com/bioassays

Technical Support: discoverx.com/support/

PathHunter Detection Kit User Manual 70-142 Rev 9