



DiscoverX

User Manual

# PathHunter<sup>®</sup> Bioassay Detection Kit

Document Number: 70-274 Revision 9



Please read this entire user manual before proceeding with the assay.  
For additional information or Technical Support, see contact information below.

## Overview

The PathHunter Bioassay Detection Kit is to be used with PathHunter Bioassay cells or PathHunter Cell Lines. The kit is easy-to-use and has been successfully run in a 96-well microplate format. The resulting signal is chemiluminescent and is read with any standard plate reader.

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  $\beta$ -galactosidase ( $\beta$ -gal) fragments. The larger  $\beta$ -gal fragment is called Enzyme Acceptor (EA) and the smaller fragment is termed Enzyme Donor (ED; also known as ProLabel® or ProLink™). 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  $\beta$ -gal, and therefore must be used in conjunction with cells that express both components in order to obtain a detectable signal.

## Materials Provided and Storage Conditions

Catalog Number	93-0933E	93-0933	93-0933L
Number of Plates	2 Plates	10 Plates	100 Plates
96-well, No. of data points	~200	~1,000	~10,000
Kit Components	Volume in Each Bottle (mL)		
Detection Reagent 1	3	15	100
Detection Reagent 2	12	60	400
<b>Total Kit Volume</b>	<b>15</b>	<b>75</b>	<b>500</b>

Upon receipt, store reagents at  $-20^{\circ}\text{C}$ , or at  $-80^{\circ}\text{C}$  for long-term storage up to the indicated shelf life of that component.

Thaw reagents at room temperature before use. Thawed reagents are stable for 4 days when stored at  $2-8^{\circ}\text{C}$ .

The reagents can tolerate up to three freeze-thaw cycles with no impact on performance.



## Additional Materials Needed

The following procedure details the assay detection protocol to be used with PathHunter Bioassay cells.

Required Materials
PathHunter Bioassay Cells
Pipettes and pipette tips
Multimode or luminescence plate reader
Disposable reagent reservoir (Thermo Scientific, Cat. No. 8094 or similar)

## Assay Detection Protocol

The following procedure details the assay detection protocol to be used with PathHunter Bioassay Cells, for assays to be run in 96-well plates. Please refer to your Bioassay kit user manual for specific assay preparation details.

- Using a multichannel pipette, add 10 µL of Detection Reagent 1 to each well of the assay plate.  Do not pipette up and down in the vial to mix or vortex plates.
- Incubate the plate at room temperature for 15 minutes in the dark.
- Using a multichannel pipette, add 40 µL of Detection Reagent 2 to each well of the assay plate.  Detection reagents are light sensitive, thus incubation in the dark is necessary.
- Incubate the plate at room temperature for 1 hour in the dark.
- Read samples on a standard luminescence plate reader at 0.1 to 1 second/ well for photomultiplier tube readers or 5 to 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 detectors collect signal from all wavelengths. Some instrument manufacturers may include a cutoff filter at high wavelengths, but usually no wavelength setting is used for luminescence readout.
- Data analysis can be performed using your choice of statistical analysis software (e.g. GraphPad Prism, Molecular Devices Softmax Pro, BioTek Gen5, Microsoft Excel, etc.).

## Supplemental Information

Instrument Compatibility Chart	
Compatible with any luminometer. Select examples indicated below.	
Berthold Technologies: Mithras LB940, CentroLIAPc	Molecular Devices: SpectraMax M3/ M4/M5/M5e, FlexStation 3, SpectraMax L
BioTek: Synergy 2, Synergy Neo2, Synergy H1, Synergy HTX, Cytation	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

\*For other instruments not listed here, please use the information below to contact Technical Support.

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