DiscoverX

User Manual

PathHunter® Bioassay ED Detection Kit

Chemiluminescent Detection

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

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Overview

The PathHunter Bioassay ED Detection Kit is to be used with PathHunter Bioassays cells overexpressing ProLabel®/ ProLink™-tagged (PL/PK-tagged) reporter or target proteins, but not expressing any EA. The Bioassay ED Detection reagent itself contains the complementary EA fragment. The kit is easy-to-use and can be run in both 96-well and 384-well microplate formats. The resulting signal is chemiluminescent and is read with any standard plate reader.

PathHunter products utilize the Enzyme Fragment Complementation (EFC) technology which involves 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 a detection substrate to generate a chemiluminescent signal.

Materials Provided

Catalog Number	**93-1043E**	**93-1043**
96-Well Plate (Data Points)	200	1,000
Kit Components	**Volume in Each Bottle**	
Detection Reagent 1 (mL)	6	30
Detection Reagent 2 (mL)	20	100
Detection Reagent 3 (mL)	5	25

Storage Conditions

Upon receipt, store reagents at or below -20°C. Thaw reagents at room temperature before use. After thawing, reagents can be stored for up to 7 days at 4°C. The reagents can tolerate up to three freeze-thaw cycles with no impact on performance.

Additional Materials Required

Materials	**Ordering Information**
PathHunter Bioassay cells expressing ED-tagged reporter or target proteins	
Single and multichannel micro-pipettors and pipette tips (10 µL – 1000 µL)	
Disposable Reagent Reservoir	Thermo Fisher Scientific, Cat. No. 8094 or similar
Multimode or luminescence reader	discoverx.com/instrument-compatibility

Assay Detection Protocol

The following procedure details the Bioassay ED Detection protocol in 96-well format for PathHunter Bioassay cells. The Assay Detection Protocol assumes that the wells contain 110 µL per well when the compound incubation step is concluded. The actual volume present in the well will depend on the optimal assay protocol specified for the Bioassay in use. Therefore, please refer to your Bioassay user manual for specific assay preparation details. Some detection reagents must be pre-mixed 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.

Assay Reagents	96-Well Plate Volume per Well	384-Well Plate Volume per Well
AssayComplete™ Cell Plating Reagent (µL)	100	20
Ligand (µL)	10	5
Detection Reagent 1 (µL)	20	5
Working Detection Solution (µL)	100	25

Working Detection Solution for 96-Well Format		
Components	**Volume Ratio**	**Volume per Plate (mL)**
Detection Reagent 2	4	10
Detection Reagent 3	1	2.5
Total Volume		**12.5**

Do not store pre-mixed working detection solution for more than 24 hours.

1. Add 20 µL of Detection Reagent 1 to all Bioassay wells on the assay plate.
2. Incubate the plate at room temperature for 15 minutes in the dark.
3. Prepare a stock of Working Detection Solution in a 15 mL polypropylene tube or reagent reservoir by mixing 4-parts of Detection Reagent 2 and 1-part of Detection Reagent 3.
4. Add 100 µL of the Working Detection Solution to all Bioassay wells on the assay plate.
5. Incubate the assay plate for 1 hour at room temperature in the dark. It's possible that the Bioassay in use was qualified using a different incubation time. Please refer to the Bioassay-specific user manual to determine the optimal incubation time recommended for the Bioassay in use.
6. 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.
7. 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.).

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