DiscoverX

User Manual

PathHunter® ProLabel®/ProLink™ Detection Kit

Chemiluminescent Detection Using Enzyme Fragment Complementation

Catalog No. 93-0812 Series

Please read entire booklet before proceeding with the assay.
For additional information or Technical Support see contact information below.

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Overview

The PathHunter ProLabel®/ProLink™ Detection Kit is to be used with PathHunter Cell Lines or cell lines made with ProLabel/ProLink (PL/PK) expression or cloning vectors. The kit is easy-to-use and has been successfully run in both 96-well and 384-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 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 contain the EA fragment of β-gal, and therefore must be used in conjunction with cells that express PL or PK in order to obtain a detectable signal.

Materials Provided and Storage Conditions

Catalog Number	**93-0812**	**93-0812L**
Number of Plates	**10 Plates**	**100 Plates**
96-well, No. of data points	~1,000	~10,000
384-well, No. of data points	~4,000	~40,000
Kit Components	**Volume in Each Bottle**	
EA Reagent	20 mL	200 mL
Lysis Buffer	20 mL	200 mL
Substrate Reagent	80 mL	800 mL
Positive Control Peptide (ED)	200 µL	1 mL

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 Needed

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

Assay Detection Protocol

The following procedure details the PL/PK assay detection in 384-well format for 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. Please refer to the Supplemental Information section for Expression Testing Protocol, which also includes a protocol for using Positive Control Peptide.

Working Detection Solution (PL/PK) for 384-well Format		
Components	Volume Ratio	Volume per Plate (mL)
EA Reagent	1	2
Lysis Buffer	1	2
Substrate Reagent	4	8
Total Volume		**12**

Do not substitute Lysis Buffer with alternative buffers. It is formulated for optimal assay performance.

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

1. Prepare a stock of Working Detection Solution (PL/PK) in a 15 mL polypropylene tube or reagent reservoir by mixing 1 part of EA Reagent, 1 part of Lysis Buffer, and 4 parts of Substrate Reagent.
2. Add 30 µL of Working Detection Solution (PL/PK) to all wells of the assay plate.

For 96-well format, prepare the Working Detection Solution (PL/PK) using the same volume ratio, add 100 µL to all wells of the assay plate.

3. Incubate assay plate for 1 hour at room temperature in the dark.
4. 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.
5. 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.

Expression Testing Protocol

Total EFC detection assays involve adding an excess of one EFC component (EA) to lysed cells to complement an ED (e.g. ProLabel®, ProLink™) tagged fusion protein allowing the determination of relative levels of expression. Total EFC detection assays may be useful for intermediary characterization, troubleshooting or characterization in the absence of a well characterized functional ligand.

The following protocol can be used to test for expression of Target-ED in your cells in 384-well format.

Confirm Expression of the Target-ED Fusion Protein

1. Assay Plate Preparation

   a. Prepare a white-walled (white or clear bottom) 384-well tissue culture treated assay plate as follows:

      i. Leave 8 wells empty (no cells) for testing the +ED (i.e. Positive Control Peptide) assay positive control.

      ii. Add 2,500 Target-ED expressing cells per well, in 20 µL cell culture medium to 8 replicate wells.

      iii. (Optional negative control) add 2,500 native cells (not expressing Target-ED) per well in 20 µL cell culture medium to 8 replicate wells.

   b. Place the seeded plate in a 37°C, 5% CO_2 humidified incubator overnight prior to detection.

   Native cell negative control can be helpful to determine activity generated by the host cell used in the cell assay.

2. Prepare Working Solutions and Controls

   a. Create two separate working solutions (one +EA and one –EA) with per well volumes indicated in the table below.

   Optional: Can be run on the same day if the number of cells on a loaded plate is doubled.

   b. For the +ED positive control wells (see Step 1.a.i), prepare 20 µL per well of a 1:4 diluted solution of ED (Positive Control Peptide) in PBS [5 µL/well ED and 15 µL/well PBS].

   Create positive control and working solutions just prior to performing the detection assay.

384-Well Format (µL/well)						
To Detect	**Working Solutions**	**EA Reagent**	**PBS**	**Cell Lysis Buffer**	**Substrate Reagent**	**Total per Well**
Signal: Target-ED	+EA Working Solution	5	0	5	20	30
Background: Target-ED & Reporter-EA	-EA Working Solution	0	5	5	20	30

3. Reagent Additions and Detection

    a. For the +ED positive control wells (see 1.a.i), add 20 µL of 1:4 diluted solution of ED per well.

    b. Add 30 µL +EA Working Solution to 4 of the 8 replicates. Add 30 µL -EA Working Solution to the other 4 replicates for the following assay conditions to be tested:

        i. The Target-ED expressing cell line

        ii. Native cells not expressing Target-ED (optional)

        iii. +ED assay positive controls

For 96-well format, prepare the Working Detection Solution (PL/PK) using the same volume ratio, add 100 µL Working Solution to wells containing cells with 100 µL of media.

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

5. 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 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.

6. 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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