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ProLink™ Detection Kit

Chemiluminescence Detection

User Manual: 92-0006

Simple Solutions for Complex Biology

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SYNERGY™ PRODUCTS

Synergy™ Products have been specifically developed for use with DiscoveRx's cell lines and assays. Please refer to page 9 for additional products and contact us if you have further questions.

INTENDED USE

The ProLink™ Detection Kit, a member of the Synergy Product Line, is used to confirm expression of ProLink-tagged proteins. ProLink (PK or ED) is a component of DiscoveRx's β-gal complementation technology and is used to engineer all PathHunter® protein-protein interaction cell lines, including β-Arrestin GPCR cell lines, Receptor Tyrosine Kinase cell lines, Nuclear Hormone Activation cell lines and others. The assay has been validated for use in both 96-well and 384-well microplate formats. This kit is a companion product for DiscoveRx's PathHunter® Product Line.

TECHNOLOGY PRINCIPLE

DiscoveRx's proprietary Enzyme Fragment Complementation (EFC) technology is a homogeneous, non-radioactive detection technology based on two genetically engineered fragments - a large protein fragment (Enzyme Acceptor, EA) and a small peptide fragment (Enzyme Donor, ED) complementing to form an active β-galactosidase enzyme that hydrolyzes the substrate to provide a chemiluminescent signal.

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.

MATERIALS PROVIDED

Product Code		**92-0006**
Number of Tests (384-well)		**800**
Item	**Description**	**Volumes**
1	EA Reagent	4 mL
2	Dilution Buffer	4 mL
3	Substrate Reagent 1	1.6 mL
4	Substrate Reagent 2*	0.32 mL
5	Cell Assay Buffer	10.1 mL
6	Positive Control	500 µL

NOTE:
*Centrifuge vial before opening to maximize recovery.

RELATED PRODUCTS

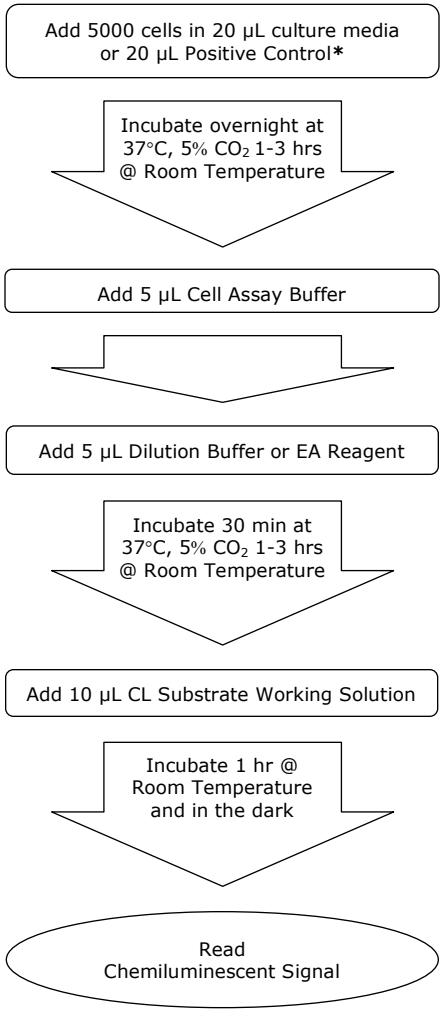
CELL LINES	**PART NUMBER**
PathHunter® β-Arrestin GPCR Cell Lines	Many*
cAMP Hunter™ GPCR Cell Lines	Many*
PathHunter® β-Arrestin Parental Cell Lines	Many*
PathHunter® Nuclear Hormone Receptor Activation Assays	Many*
PathHunter® Receptor Tyrosine Kinase Cell Lines	Many*
PathHunter® Translocation Parental Cell Lines	Many*

REAGENTS	
PathHunter® Flash Detection Kit	93-0247
ProLink™ Cloning Vector Bundle	93-0173
ProLabel™ Cloning Vectors	Many*
ProLink™ Detection Kit	92-0006
ProLabel™ Detection Kit	93-0180
Forskolin	92-0005

*Please visit **www.discoverx.com** or contact us for more information.

QUICK-START PROCEDURE

In a White-Walled 384-well plate perform the following:



***There is no need to incubate the positive control overnight. The positive control can be used on the day of the assay. The overnight incubation is to allow the cells to properly attach to the plate.

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

The following additional materials are required:

Equipment	Materials
• Pipettes and pipette tips • 384-well or 96-well microplates (white-walled with clear bottom, or solid white walled) • Multi-mode or luminescence plate reader	• DiscoveRx cell lines expressing PK-tagged proteins

REAGENT PREPARATION

EA Reagent: Ready to use.

CL Substrate Working Solution:

Prepare **CL Substrate Working Solution** by mixing 1 part Substrate Reagent 2 with 5 parts Substrate Reagent 1 and 19 parts Cell Assay Buffer. Once prepared, the working solution is stable for at least 24 hours at room temperature with no impact on assay performance.

NOTE:
Do not substitute Cell Assay Buffer with alternative buffers. It is formulated for optimal assay performance.

Positive Control: Ready to use.

GUIDELINES FOR USE

The assay can be run in 384-well or 96-well microplates. Please refer to the Assay Procedure section and Quick Start Guide of the product insert. Samples can be read on any multi-mode or dedicated microplate reader that is properly configured for detection of luminescence.

ASSAY PROCEDURE

The following table outlines the volumes and procedures for running the ProLink Detection Kit. Please also refer to the Quick Start Guide (page 8).

Steps	Volumes (96-well Protocol)	Volumes (384-well Protocol)
Step 1: Add cells or positive control	20000 cells in 80 µL culture media or 80 µL positive control*	5000 cells in 20 µL culture media or 20 µL positive control*
Step 2: Incubate	Incubate overnight at 37°C, 5% CO_2	
Step 3: Add Cell Assay Buffer	20 µL Cell Assay Buffer	5 µL Cell Assay Buffer
Step 4: Add EA Reagent	20 µL EA Reagent or 20 µL Dilution Buffer**	5 µL EA Reagent or 5 µL Dilution Buffer**
Step 5: Incubate	Incubate at 37°C, 5% CO_2 for 30 minutes	
Step 6: Add Substrate	40 µL CL Substrate Working Solution	10 µL CL Substrate Working Solution
Step 7: Incubate and Read Samples	Incubate in the dark at room temperature for 1 hour and read on any standard luminometer.	

*There is no need to incubate the positive control overnight. The positive control can be used on the day of the assay. The overnight incubation is to allow the cells to properly attach to the plate.

**It is highly recommended that the assay is run with and without the EA Reagent to determine expression of PK-tagged proteins. Use the Dilution Buffer for the "without EA Reagent" wells.

REPRESENTATIVE DATA

Example: Confirm Expression of Orphan GPCRs

DiscoveRx's Orphan GPCR Cell Lines, GPR88 (DiscoveRx, Cat. #93-0357C2A) and ADMR (DiscoveRx, Cat. #93-0403C2) were analyzed using this kit to confirm expression of the respective GPCR-PK fusion proteins.

	Dilution Buffer RLU	EA Reagent RLU	EA/Dilution Buffer Ratio
GPR88	464	8748	19
ADMR	501	1911	4

These data demonstrate that GPR88-PK and ADMR-PK are expressed at abundant levels because of the significant Raw Luminescence Units (RLU) difference between the dilution buffer and the EA reagent. In general, the ratio of EA Reagent/Dilution Buffer RLU values for any particular cell line expressing a PK-tagged protein should be ≥ 2.