PathHunter® Anti-ProLabel®/ProLink™ Antibody

Materials Provided

Catalog Numbers: 92-0010, PathHunter Anti-PL/PK Antibody

Size: 50 µg
Quantity Supplied: 1 vial, 100 µL at 0.5 mg/mL

Description

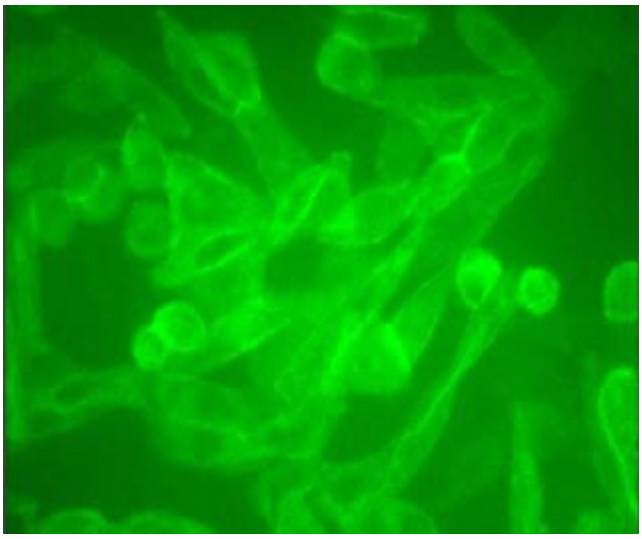
The Anti-ProLabel/ProLink antibody has been specifically developed for use with PathHunter cell lines expressing the small enzyme donor tag enhanced ProLabel (PL) or ProLink (PK). This antibody, subclass $IgG_{2a}(\kappa)$, was developed from a mouse hybridoma and recognizes the PL or PK fragment of β-galactosidase.

Product Information

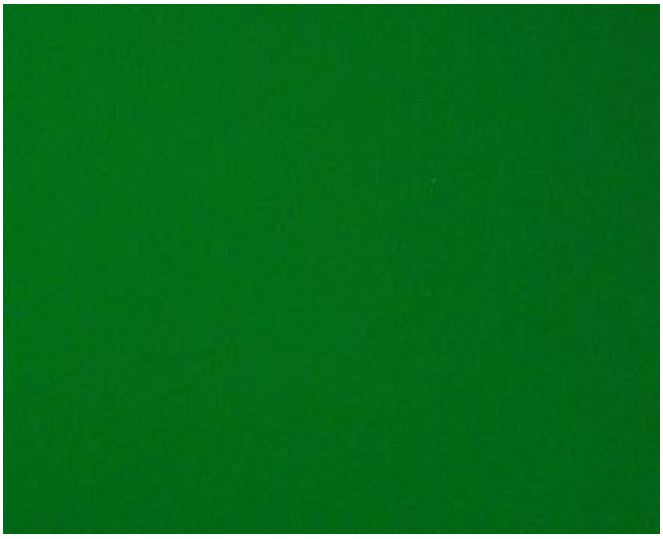
Molecular Weight: ~150 kDa
Source: Mouse monoclonal antibody
Storage Conditions: Store at -20°C. Can be stored at 4°C for up to 5 weeks.
Applications: Immunocytochemistry (1:100 to 1:500)
Specificity: Synthetic peptide corresponding to residues in the enzyme donor (ED), PL or PK fragments of β-galactosidase, used as immunogen.
Formulation: Formulated at 0.5 mg/mL in 10 mM phosphate buffer, 0.14 M NaCl, and 0.05% Sodium Azide, pH 7.2

Application Data

Immunostaining Analysis



Immunocytochemistry using anti-PL/PK antibody and PathHunter® CHO-K1 GPR92 β-Arrestin Cell Line (Cat. No. 93-0372C2). PK1 labeled.



Native CHO-K1 Cells (control)

Instructions for Use

Immunostaining Analysis

This procedure can be used to visualize engineered proteins fused to the small enzyme donor (PL or PK) fragment of β-galactosidase. The steps outlined below should be used as a starting point for labeling proteins at the membrane, in the endosome, in the cytosol, or in the nucleus of cells. Optimization of the fixation and permeabilization conditions may be required to maximize label intensity for different types of proteins. Appropriate negative controls should be run in parallel (e.g. native cells lacking any PK- or PL-fusion protein expression).

Materials

- DiscoverX cells containing a PL or PK tagged protein
- Formaldehyde Solution, 16% (Paraformaldehyde Solution) EM Grade (Electron Microscopy Sciences, Inc. Hatfield, PA, Cat. No. 15710-S)
- Phosphate-buffered saline (PBS), pH 7.2 (Sigma-Aldrich, Cat. No. P5119).
- Bovine serum albumin (BSA) (Sigma-Aldrich, Cat. No. A-7888)
- Saponin (Sigma-Aldrich, Cat. No. S7900)
- PathHunter Anti-PL/PK Antibody (DiscoverX Cat. No. 92-0010)
- FITC conjugated secondary antibody (secondary anti-Mouse IgG at 1 to 2.5 µg/mL diluted in 1% BSA plus PBS)
- Anti-Fade Solution (VECTASHIELD HardSet Mounting Medium with DAPI; Vector Labs, Cat. No. H-1500)

Procedure

1. Seed DiscoverX cells overnight.
2. Fix cells in 3.7% formaldehyde for 15 to 20 minutes at room temperature.
3. Wash cells by rinsing 3 times in PBS for 2 minutes each.
4. Permeabilize cells with 0.2% Saponin for 20 minutes at room temperature.
5. Wash cells by rinsing 3 times in PBS for 2 minutes each.
6. Treat cells with PBS plus 1% BSA for 10 minutes to block un-reacted formaldehyde.
7. During the 10-minute treatment in Step 6, prepare a working solution of Anti-PL/PK Antibody at 1 to 5 µg/mL in PBS plus 1% BSA.
8. Aspirate PBS plus 1% BSA from cells and immediately add 200 µL of working solution of Anti-PL/PK Antibody from Step 7.
9. Incubate cells for 1 hour at room temperature.
10. During the 1 hour incubation in Step 9, prepare a working solution of FITC-conjugated Anti-Mouse IgG at 1 to 2.5 µg/mL in PBS plus 1% BSA.
11. Wash cells by rinsing 3 times in PBS for 2 minutes each.
12. Aspirate PBS and immediately add 200 µL of working solution of FITC-conjugated Anti-Mouse IgG from Step 10.
13. Incubate cells for 20 minutes at room temperature.
14. Wash cells by rinsing 4 times in PBS for 2 minutes each.
15. Aspirate PBS and wet mount cells with anti-fade solution. Put a small drop of anti-fade solution on a glass slide. Use forceps to pick up coverslip and aspirate any excess liquid. Place coverslip, cell side down, onto glass slide. Aspirate any excess anti-fade solution from edges of the coverslip. Seal edges.
16. Examine staining under a fluorescence microscope.

For additional information or Technical Support, see contact information below.

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