PathHunter® Anti-EA Antibody

Materials Provided

Catalog Number: 92-0246 Series
Sizes: 50 µg or 100 µg (L size)

Description

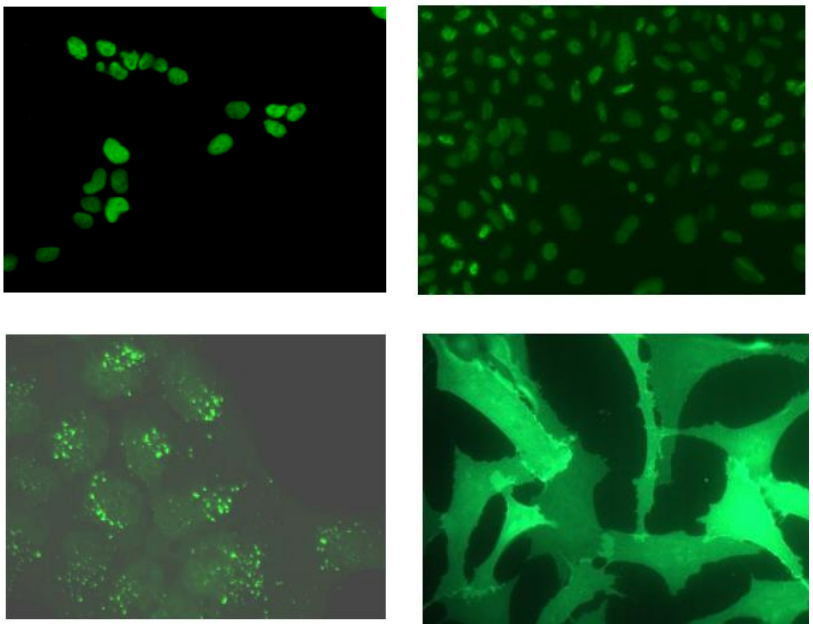
The anti-EA antibody has been specifically developed for use with PathHunter cell lines expressing the large enzyme acceptor (EA) fragment. This antibody, subclass $IgG_{2a}(\kappa)$, was developed from ascites of a mouse hybridoma and recognizes the EA fragment of β-galactosidase.

Product Information

Molecular Weight: ~150 kDa
Source: Mouse monoclonal antibody
Storage Conditions: Store at -20°C.
Applications: Western Blot (1:5000), Immunocytochemistry (1:1000), Dot Blot (1:5000)
Specificity: Synthetic peptide corresponding to residues in the enzyme acceptor (EA) fragment of β-galactosidase.
Formulation: Formulated at 2.0 mg/mL in 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.02% sodium azide

Application Data

Immunostaining Analysis



Immunocytochemistry co-localization studies using anti-EA antibody and PathHunter® EA parental cell lines (top-left) U2OS NUC-EA (cat. no. 93-0178), (top-right) CHO-K1 NUC-EA (cat. no. 93-0004), (bottom-left) U2OS ENDO-EA (cat. no. 93-1102C3, and (bottom-right) U2OS MEM-EA (cat. no. 93-1101C3).

Co-localization of anti-EA staining (green) in the cells indicates expression of EA-labeled proteins (NUC, ENDO, and MEM) in the nucleus (top images), in the endosome, or at the cell membrane, respectively.

Instructions for Use

Immunostaining Analysis

This procedure describes the fixation and labeling conditions that are optimal for visualizing the large EA fragment of β-galactosidase in cells. 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.

Materials

- DiscoverX cells containing expressed EA
- 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).
- Tris-Buffered Saline (TBS) (Dako, cat. no. S3001)
- Blocking solution – BLOTTO (3% Nonfat dried milk, 0.1% Triton X-100 (Sigma-Aldrich, cat. no. T-9284) in TBS
- PathHunter® Anti-EA Antibody (DiscoverX cat. no. 93-0246)
- Appropriate secondary antibody (e.g. goat anti-Mouse IgG (H+L), Alexa-488 conjugated secondary antibody (Thermo Fisher Scientific; cat. no. A11029)
- Anti-Fade Solution (VECTASHIELD HardSet Mounting Medium with DAPI; Vector Labs, cat. no. H-1500)

Procedure

1. Culture cells in a 6-well dish with 18 mm square coverslip overnight.
2. Aspirate media and add 3.7% formaldehyde fixative for 15 to 20 minutes at room temperature.
3. Wash cells with PBS 3 times for 2 minutes.
4. Add 1 mL blocking solution to wells and incubate for 20 minutes.
5. Aspirate blocking solution and incubate cells with PathHunter anti-EA Antibody (diluted with the blocking solution) for 45 to 60 minutes.
6. Wash cells with PBS 3 times for 2 minutes each.
7. Aspirate blocking solution and incubate cells with secondary antibody (diluted with the blocking solution) for 20 to 30 minutes in the dark.
8. Wash cells with PBS for 3 times for 2 minutes.
9. Aspirate PBS and wet mount 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.

For order placement or technical support, please call 1.866.448.4864 (North America) or +44.121.260.6142 (Europe) or email info@discoverx.com. For additional information, please visit discoverx.com.

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