Technical Data Sheet

Alexa Fluor® 488 Mouse anti-TIE2 (pY1102)

Product Information

Material Number: 560053
Alternate Name: TEK, VMCM, CD202b
Size: 50 Tests
Vol. per Test: 20 µl
Clone: K93-754
Immunogen: Phosphorylated Human TIE2 Peptide
Isotype: Mouse (BALB/c) IgG2a, κ
Reactivity:

QC Testing: Human
Predicted Reactivity: Mouse

Storage Buffer:
Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

TIE2 (Tyrosine kinase with Immunoglobulin-like and EGF-like domains 2), also known as TEK (Tunical Endothelial Kinase), is an endothelial and hematopoietic cell-specific receptor tyrosine kinase (RTK) that is critical to the development and maintenance of the vasculature and highly conserved among vertebrate species. The angiopoietins are ligands of TIE2, and the abnormal vascular growth that often occurs in solid tumors is a result of disruptions in the coordinated actions of the angiopoietins, TIE2, and the closely related TIE1 RTK. Upon activation by angiopoietins, TIE2 autophosphorylates at least 2 tyrosines in its protein kinase domain and at least 3 tyrosines in its C-terminal tail. Phosphorylation at tyrosine 1102 (Y1102) leads to activation of the Akt signal transduction pathway, enhancing endothelial cell survival. The phosphorylated Y1102 also recruits and activates the src homology 2 domain-containing adaptor proteins Grb2 and ShcA, affecting endothelial cell adhesion and motility.

The K93-754 monoclonal antibody recognizes the phosphorylated Y1102 in the C-terminal tail of activated TIE2. The orthologous phosphorylation site in mouse TIE2 is Y1100.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Analysis of TIE2 (pY1102) in human vascular endothelium. EA-hy 926 cells (Edgell, McDonald, Graham, 1983)) were serum starved overnight, detached using 1X trypsin, washed, resuspended in serum-free DMEM and rested for 20 minutes at 37°C, and then either left unstimulated (open histogram) or stimulated with 1 mM Pervanadate (Sigma Cat. No. S6508, shaded histogram) for 20 minutes at 37°C (left figure). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 488 Mouse anti-TIE2 (pY1102, Cat. No. 560053). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system. The specificity of mAb K93-754 was confirmed by western blot using unconjugated antibody, at 0.25, 0.125 and 0.063 µg/ml (lanes 1, 2, and 3, respectively) on lysates from control (left panel) and Pervanadate-treated (right panel) EA-hy 926 cells (right figure). TIE2 (pY1102) is identified as a band of 160 kDa in the treated cells.
Recommended Assay Procedure:
Either BD Cytofix™ fixation buffer or BD Phosflow™ Fix Buffer I may be used for cell fixation.

Suggested Companion Products

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<th>Name</th>
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<td>Stain Buffer (BSA)</td>
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Product Notices
1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use $1 \times 10^6$ cells in a 100-µl experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References
Edgell C-JS, McDonald CC, Graham JB. Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc Natl Acad Sci U S A. 1983; 80:3734-3737. (Methodology: Controls)