



cell signaling solutions

Certificate of Analysis

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Anti-Flt-1, clone BK302

(mouse monoclonal IgG_{1κ})

Catalog # 05-696

Lot # 24325

Immunogen: GST fusion protein corresponding to residues 1251-1338 of human Flt-1.

Specificity: Recognizes human Flt-1, Mr ~180kDa.

Species Cross-reactivity: Human. Other species not tested.

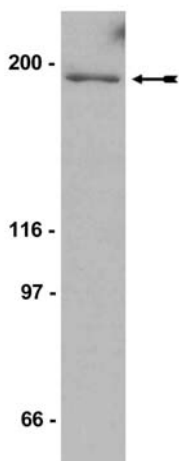
Formulation: 200μg of protein G purified mouse IgG_{1κ} in 200μl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5-2μg/ml of this lot detected Flt-1 in Human umbilical vein endothelium (HUVEC) membrane preps.



Immunoblot Analysis

HUVEC membrane cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Flt-1 (2μg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Flt-1 (180kDa).

Additional Research Applications

Immunoprecipitation: NOT recommended.

General References:

1. Peters, K.G., *et al.*, Proc. Nat. Acad. Sci. **90**: 8915-8919, 1993.
2. Ito, N., *et al.*, J. Biol. Chem. **273**: 23410-23418, 1998.
3. Rahimi, N., *et al.*, J. Biol. Chem. **275**: 16986-16992, 2000.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a membrane fractions sample (cell lysis buffer: 20mM Tris-HCl, pH 8; 5mM EDTA, 1mM EGTA, 1mM PMSF, 5ug/ml Leupeptin, 5ug/ml Aprotinin, 1 mM Na_3VO_4) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 $\mu\text{g}/\text{ml}$ of anti-Fit-1**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, 1:5000 dilution was used) in TBST-MLK for 1 hour at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 10 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).