

## Anti-Human Flt-1 C-Terminus (180kDa)

(chicken polyclonal IgY)

Catalog # 06-670

Lot # 16231

**Immunogen:** KLH conjugated, 22 residue synthetic peptide (DDVRYVNAFKFMSLERIKTFEE) corresponding to amino acids 1209-1230 of human-Flt-1.

**Specificity:** Recognizes human Flt-1 of 180 kDa.

**Species Cross-reactivity:** Not determined. Based on sequence homology (22/22), likely to cross-react with mouse and rat.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

**Formulation:** 200mg of IgY, purified by PEG and ammonium sulfate precipitation, in 200ml of sterile PBS, 0.05% sodium azide. Frozen solution.

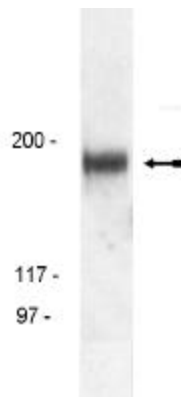
**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing

**Immunoblot Analysis:** 1µg/ml of this lot detected recombinant Flt-1 in a membrane preparation from Hi5 insect cells expressing recombinant Flt-1.

**Immunoprecipitation:** Not recommended.

**Immunocytochemistry:** Not recommended.



#### Immunoblot Analysis

Hi5 insect cells expressing recombinant Flt-1 membrane prep cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Human Flt-1 CT (1µg/ml). Proteins were visualized using a goat anti-chicken secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Flt-1 (180kDa).

**Background:** Flt-1 (fms-like tyrosine kinase-1) is a member of the *flt* family of receptor tyrosine kinases that also includes KDR/*flk-1* and *flt-4*. The *flt* family encodes receptors for VEGF (vascular endothelial growth factor) that are characterized by seven Ig-like loops within their extracellular domain and a split kinase domain within the cytoplasmic domain. VEGF is an important angiogenic factor of many solid tumors and is also involved in the differentiation of endothelial cells. It has been reported that *flt-1* receptor dimerization precedes phosphorylation after VEGF binding.

#### General References:

- Barleon, B., *et al.*, *J. Biol. Chem.* **272**: 10382-10388, 1997.  
Cunningham, S.A., *et al.*, *Biochem. Biophys. Res. Commun.* **231**: 596-599, 1997.  
Wakiya, K., *et al.*, *J. Biol. Chem.* **271**: 30823-30828, 1996.  
Barleon, B., *et al.*, *Blood* **87**: 3336-3343, 1996.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a membrane preparation and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1ng/ml of  $\alpha$ -Human Flt-1 CT**, diluted in freshly prepared PBS-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  $\alpha$ -chicken** HRP conjugated IgG, 1:3000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).