
Certificate of Analysis

Anti-Flt1, CT

(chicken polyclonal IgY)

Catalog # 06-670

Lot # 32206

Immunogen: KLH conjugated, 22 residue synthetic peptide (DDVRYVNAFKFMSLERIKTFEE) corresponding to amino acids 1209-1230 of human-Flt1.

Specificity: Recognizes human Flt1, Mr 180kDa.

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

Formulation: 200µg of chicken IgY, purified by PEG and ammonium sulfate precipitation, in 200µl of storage buffer (sterile PBS, 0.05% sodium azide). Frozen solution.

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.**

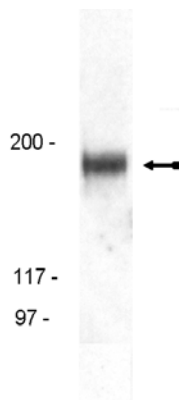
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected recombinant Flt1 in 200ng active Flt1 (Catalog # 14-562). A previous lot detected Flt1 in a membrane preparation from Hi5 insect cells expressing recombinant Flt1.

Immunoprecipitation: Not recommended.

Immunocytochemistry: Not recommended.



Immunoblot Analysis

Representative blot from a previous lot. Hi5 insect cells expressing recombinant Flt1 membrane prep cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Flt1, CT (1µg/ml). Proteins were visualized using a goat anti-chicken secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Flt1 (~180kDa).

General References:

1. Barleon, B., *et al.*, *J. Biol. Chem.* **272**: 10382-10388, 1997.
2. Cunningham, S.A., *et al.*, *Biochem. Biophys. Res. Commun.* **231**: 596-599, 1997.
3. Wakiya, K., *et al.*, *J. Biol. Chem.* **271**: 30823-30828, 1996.
4. 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 (Catalog # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-FIt1, 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 **rabbit anti-chicken** HRP conjugated IgG, Catalog # 12-341, 1:3000 dilution was used) in PBS-MLK overnight at 4°C 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).