Rabbit (polyclonal)  
Anti-FLT3 \[pY^{842}\]  
Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: 44-1129G (10 mini-blot size)
Lot Number: See product label
Volume: 100 μL
Form of Antibody: Rabbit polyclonal immunoglobulin in Dulbecco’s phosphate buffered saline (without Mg\(^{2+}\) and Ca\(^{2+}\)), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative: 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification: Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated FLT3. The final product is generated by affinity chromatography using a FLT3-derived peptide that is phosphorylated at tyrosine 842.
Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human FLT3 that contains tyrosine 842.
Target Summary: FLT3 (Fms-like tyrosine kinase 3; also known as FLK2, STK1 (stem cell tyrosine kinase 1) or CD135) is a receptor tyrosine kinase for the hematopoietic growth factor FLT3 ligand (FL) and is expressed on both hematopoietic stem cells and progenitors. FLT3 belongs to the class III family of RTKs, which include c-Kit, CSF-R, and PDGFR and plays an important role in the proliferation, differentiation and survival of hematopoietic cells. Activating mutations of this receptor are the most common genetic abnormality seen in acute myeloid leukemia (AML), with 7% attributed to mutations in the tyrosine kinase domains. Activation of the receptor leads to tyrosine phosphorylation of a series of key adaptor proteins such as, Shc, Vav, PLC\(\gamma\), Gap, Dok, and p190RhoGAP. FLT3 is phosphorylated on several tyrosine sites in the juxtamembrane, tyrosine kinase and the carboxyl-terminal domains. Tyrosine 842, located in the tyrosine kinase domain is critical for FLT3 phosphorylation and its activation of downstream signaling proteins including Erk1/2, STAT3 and STAT5.
Reactivity: Human FLT3. Mouse (FLT3 Tyrosine 845) (90% homologous) is expected to react.
Applications: The antibody has been used for Western blotting applications.
Suggested Working Dilutions: For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage: Store at ~20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at ~20°C. For shipment or short-term storage (up to one week), 2 to 8°C is sufficient.
Expiration Date: Expires one year from date of receipt when stored as instructed.
Positive Controls Used: RS4;11 cells treated with FL (100 ng/mL) for 10 min.

This product is for research use only. Not for use in diagnostic procedures.

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Related Products: Antibodies:
- FLT3 [pY909], Cat. # 44-1131G
- AKT/PKB [pS473], Cat. # 44-621G
- ERK1/2 [pTpY185/187], Cat. # 44-680G
- FLT3 [pY955], Cat. # 44-1130G
- c-Kit [pY721], Cat. # 44-494G
- c-Kit [pY936], Cat. # 44-500G
- PDGFα[pY849]/β[pY857], Cat. # 44-1001G
- SHC [pYpY239/240], Cat. # 44-830
- SHP2 [pY542], Cat. # 44-554G
- SHP2 [pY580], Cat. # 44-558G
- STAT5 [pY694], Cat. # 44-390G
- Vav 1 [pY669], Cat. # 44-482

References:

Antibody Specificity
Extracts of RS 4;11 cells left unstimulated (1) or stimulated (2) with 100 ng/mL FL for 10 minutes were resolved by SDS-PAGE on an 8% polyacrylamide gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer for one hour at room temperature, then incubated with the FLT3 [pY842] antibody applied at 4°C in a 3% BSA-TBST buffer. After washing, the membrane was incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using a chemiluminescent method. The data show that the signal was induced by Flt stimulation. Additionally, peptide competition analysis (data not shown) indicates that only phospho-peptide corresponding to FLT3 [pY842], blocks the antibody signal, demonstrating the specificity of the antibody.

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Western Blotting Procedure

1. Lyse approximately $10^7$ cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.

2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.

3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.

4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.

5. Load 10-30 μg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.

6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.

7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.

8. Assemble the gel and membrane into the sandwich apparatus.

9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.

10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.

11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.

12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for one hour at room temperature.

13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.

14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab')2 anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat F(ab')2 anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

### Cell Lysis Buffer

**Formulation:**
- 10 mM Tris, pH 7.4
- 100 mM NaCl
- 1 mM EDTA
- 1 mM EGTA
- 1 mM NaF
- 20 mM Na$_4$P$_2$O$_7$
- 2 mM Na$_3$VO$_4$
- 0.1% SDS
- 0.5% sodium deoxycholate
- 1% Triton-X 100
- 10% glycerol
- 1 mM PMSF (made from a 0.3 M stock in DMSO)
- or 1 mM AEBSF (water soluble version of PMSF)
- 60 μg/mL aprotinin
- 10 μg/mL leupeptin
- 1 μg/mL pepstatin
- (alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

### Transfer Buffer

**Formulation:**
- 2.4 gm Tris base
- 14.2 gm glycine
- 200 mL methanol
- Q.S. to 1 liter, then add 1 mL 10% SDS

### Tris Buffered Saline

**Formulation:**
- 20 mM Tris-HCl, pH 7.4
- 0.9% NaCl

### Blocking Buffer

**Formulation:**
- 100 mL Tris buffered saline
- 3 gm Ig-free BSA
- 0.1 mL Tween 20

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