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#7021

## PathScan® Phospho-FLT3 (Tyr591) Chemiluminescent Sandwich ELISA Kit

**Species Cross Reactivity:** H  
**UniProt ID:** #P36888  
**Entrez-Gene Id:** #2322

**For Research Use Only. Not for Use in Diagnostic Procedures.**

Product Includes	Product #	Quantity	Color	Storage Temp
FLT3 Mouse mAb Coated Microwells	85498	96 tests		+4C
Phospho-FLT3 (Tyr591) Rabbit Detection Antibody	14073	1 ea	Green (Lyophilized)	+4C
Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated)	13272	1 ea	Red (Lyophilized)	+4C
Detection Antibody Diluent	13339	5.5 ml	Green	+4C
HRP Diluent	13515	5.5 ml	Red	+4C
Luminol/Enhancer Solution	84850	3 ml		RT
Stable Peroxide Buffer	42552	3 ml		RT
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml		+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml		-20C

### Description

The PathScan® Phospho-FLT3 (Tyr591) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of phospho-FLT3 (Tyr591) protein with a chemiluminescent readout. Chemiluminescent ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using a smaller sample size. A FLT3 Mouse mAb has been coated on the microwells. After incubation with cell lysates, FLT3 (phospho or nonphospho) protein is captured by the coated antibody. Following extensive washing, a phospho-FLT3 (Tyr 591) Rabbit Antibody is added to detect the captured phospho-FLT3 protein. Anti-rabbit IgG, HRP-linked antibody is then used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of phospho-FLT3 (Tyr591) protein.

\*Antibodies in this kit are custom formulations specific to kit.

### Specificity/Sensitivity

PathScan® Phospho-FLT3 (Tyr591) Chemiluminescent Sandwich ELISA Kit #7021 detects endogenous levels of phospho-FLT3 (Tyr591) in human cells. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

### Background

FMS-related tyrosine kinase 3 (FLT3, also called FLK2) is a member of the Type III receptor tyrosine kinase family, which includes c-Kit, PDGFR, and M-CSF receptors. FLT3 is expressed on early hematopoietic progenitor cells and supports growth and differentiation within the hematopoietic system (1,2). FLT3 is activated after binding with its ligand FL, which results in a cascade of tyrosine autophosphorylation and tyrosine phosphorylation of downstream substrates (3). The p85 subunit of PI3 kinase, SHP2, GRB2, and Shc have all been reported to associate with FLT3 after FL stimulation (4-6). Tyr589/591 is located in the juxtamembrane region of FLT3 and may play an important role in regulation of FLT3 tyrosine kinase activity. Somatic mutations of FLT3 consisting of internal tandem duplications (ITDs) occur in 20% of patients with acute myeloid leukemia (7).

### Background References

- Shurin, M.R. et al. (1998) *Cytokine Growth Factor Rev* 9, 37-48.
- Naoe, T. et al. (2001) *Cancer Chemother Pharmacol* 48 Suppl 1, S27-30.
- Namikawa, R. et al. (1996) *Stem Cells* 14, 388-95.
- Beslu, N. et al. (1996) *J Biol Chem* 271, 20075-81.
- Zhang, S. and Broxmeyer, H.E. (2000) *Biochem Biophys Res Commun* 277, 195-9.
- Zhang, S. et al. (1999) *J Leukoc Biol* 65, 372-80.
- Mizuki, M. et al. (2000) *Blood* 96, 3907-14.

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#7021

# PathScan<sup>®</sup> Phospho-FLT3 (Tyr591) Chemiluminescent Sandwich ELISA Kit

## ELISA Chemiluminescent (Lyophilized)

**NOTE:** Refer to product-specific datasheets for assay incubation temperature. This chemiluminescent ELISA is offered in low volume microplates. Only 50  $\mu$ l of samples or reagents are required in each microwell.

### A. Solutions and Reagents

**NOTE:** Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 0.5 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the 0.5 ml volume of reconstituted Detection Antibody to 5.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody\*:** Supplied lyophilized as a red colored cake or powder. Add 0.5 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the 0.5 ml volume of reconstituted HRP-Linked Antibody to 5.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody.
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody.
6. **Sample Diluent:** Blue colored diluent for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan<sup>®</sup> Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **Luminol/Enhancer Solution and Stable Peroxide Buffer.**

\***NOTE:** Some PathScan<sup>®</sup> ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.

### B. Preparing Cell Lysates

#### For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

#### For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10<sup>6</sup> viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

### C. Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan<sup>®</sup> Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection

of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.

3. Add 50  $\mu$ l of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
  1. Discard plate contents into a receptacle.
  2. Wash 4 times with 1X Wash Buffer, 150  $\mu$ l each time for each well.
  3. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
  4. Clean the underside of all wells with a lint-free tissue.
5. Add 50  $\mu$ l of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50  $\mu$ l of reconstituted HRP-linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50  $\mu$ l of the Working Solution to each well.
11. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. Optimal signal intensity is achieved when read within 10 min.

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