

PathScan® Phospho-FGF Receptor 3 (panTyr) Chemiluminescent Sandwich ELISA Kit

1 Kit (96 assays)



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For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H

Description: The PathScan® Phospho-FGF Receptor 3 (panTyr) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of tyrosine-phosphorylated FGFR3 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. An FGFR3 rabbit mAb has been coated on the microwells. After incubation with cell lysates, both phospho- and non-phospho-FGFR3 proteins are captured by the coated antibody. Following extensive washing, a phospho tyrosine mouse mAb is added to detect the captured tyrosine-phosphorylated FGFR3 protein. Anti-mouse 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 tyrosine-phosphorylated FGFR3 protein.

*Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: PathScan® Phospho-FGF Receptor 3 (panTyr) Chemiluminescent Sandwich ELISA Kit #64740 detects endogenous levels of tyrosine-phosphorylated FGFR3 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: Fibroblast growth factors (FGFs) produce mitogenic and angiogenic effects in target cells by signaling through cell surface receptor tyrosine kinases. There are four members of the FGF receptor family: FGFR1 (flg), FGFR2 (bek, KGFR), FGFR3, and FGFR4. Each receptor contains an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic kinase domain (1). Following ligand binding and dimerization, the receptors are phosphorylated at specific tyrosine residues (2). Seven tyrosine residues in the cytoplasmic tail of FGFR1 can be phosphorylated: Tyr463, 583, 585, 653, 654, 730, and 766. Tyr653 and Tyr654 are important for catalytic activity of activated FGFR and are essential for signaling (3). The other phosphorylated tyrosine residues may provide docking sites for downstream signaling components such as Crk and PLCγ (4,5).

Activating mutations within fibroblast growth factor receptor 3 (FGFR3) are responsible for human skeletal dysplasias including achondroplasia and the neonatal lethal syndromes thanatophoric dysplasia types I and II (6,7). Several of these same FGFR3 mutations as well as overexpression of FGFR3 proteins have also been identified somatically in human cancers, including multiple myeloma, bladder carcinoma and cervical cancer (8). Thus, FGFR3 may represent a potential target for therapy.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
FGFR3 Rabbit mAb Coated Microwells	67593	96 tests		4°C
Phospho Tyrosine Mouse Detection mAb	12982	1 each	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
Luminol/Enhancer Solution	84850	11 ml		RT
Stable Peroxide Buffer	42552	11 ml		RT
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

Low volume microplate *12 8-well modules – Each module is designed to break apart for 8 tests.

Note: This kit contains components with mixed storage temperatures. Please store this entire kit at 4°C for long term storage. Upon first use, please store each component as indicated in the chart above and on individual component labels.

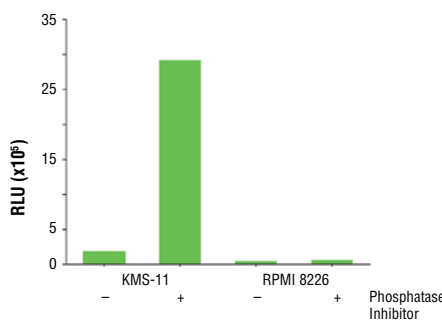


Figure 1. Constitutive tyrosine-phosphorylation of FGFR3 in KMS-11 cells lysed in the presence of phosphatase inhibitors (phospho lysate) is detected by PathScan® Phospho-FGF Receptor 3 (panTyr) Chemiluminescent Sandwich ELISA Kit #64740. In contrast, a low level of phospho-FGFR3 protein is detected in KMS-11 cells lysed in the absence of phosphatase inhibitors* (nonphospho lysate). For the FGFR3-negative RPM1 8226 cells, there is no phospho-FGFR3 detected by this ELISA kit in either presence or absence of phosphatase inhibitors*. Immediate light generation with chemiluminescent substrate is shown in the top figure. *Phosphatase inhibitors include sodium pyrophosphate, β-glycerophosphate, and sodium orthovanadate.

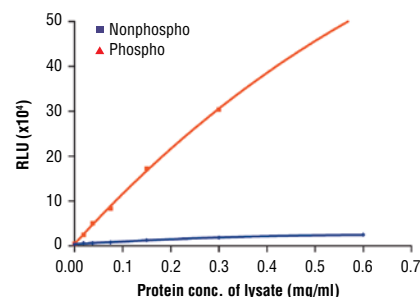


Figure 2. The relationship between protein concentration of phospho or nonphospho lysates and immediate light generation with chemiluminescent substrate as detected by the PathScan® Phospho-FGF Receptor 3 (panTyr) Chemiluminescent Sandwich ELISA Kit is shown. Unstarved KMS-11 cells were cultured (0.8 x 10⁶ cells/ml) and lysed with or without addition of phosphatase inhibitors to the lysis buffer (phospho or nonphospho lysate, respectively).

U.S. Patent No. 5,675,063

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PathScan® Chemiluminescent Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

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

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- 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 full 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.
- 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 full 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.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (5.5 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (5.5 ml provided).
- Sample Diluent:** Blue colored diluent for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 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.
- Luminol/Enhancer Solution and Stable Peroxide Buffer**

*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.

B Preparing Cell Lysates

For adherent cells.

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- 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.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- Microcentrifuge for 10 min (14,000 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

- Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
- Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
- Sonicate lysates on ice.
- Microcentrifuge for 10 min (14,000 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

- 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.
- Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® 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.
- Add 50 µl 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.
- Gently remove the tape and wash wells:
 - Discard plate contents into a receptacle.
 - Wash 4 times with 1X Wash Buffer, 150 µl each time for each well.
 - 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.
 - Clean the underside of all wells with a lint-free tissue.
- Add 50 µl 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.
- Repeat wash procedure (Section C, Step 4).
- Add 50 µl 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.
- Repeat wash procedure (Section C, Step 4).
- Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
- Add 50 µl of the Detection Reagent Working Solution to each well.
- 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.*

Background References:

- (1) Powers, C.J. et al. (2000) *Endocr Relat Cancer* 7, 165-97.
- (2) Reilly, J.F. et al. (2000) *J Biol Chem* 275, 7771-8.
- (3) Mohammadi, M. et al. (1996) *Mol Cell Biol* 16, 977-89.
- (4) Mohammadi, M. et al. (1991) *Mol Cell Biol* 11, 5068-78.
- (5) Larsson, H. et al. (1999) *J Biol Chem* 274, 25726-34.
- (6) Wilkie, A.O. et al. (2002) *Am J Med Genet* 112, 266-78.
- (7) Yamashita, A. et al. (2014) *Nature* 513, 507-11.
- (8) Miyake, M. et al. (2007) *Biochem Biophys Res Commun* 362, 865-71.