

**#3476** Store at -20°C

# Phospho-FGF Receptor (Tyr653/654) (55H2) Mouse mAb

- Small 100 µl (10 Western mini-blot)
- Large 300 µl (30 Western mini-blot)



**Orders** ■ 877-616-CELL (2355)  
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**Support** ■ 877-678-TECH (8324)  
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Entrez-Gene ID** # 2260  
**Swiss-Prot Acc.** # P11362

Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W Transfected	H, (M, R)	120 to 145 kDa	Mouse	IgG2b

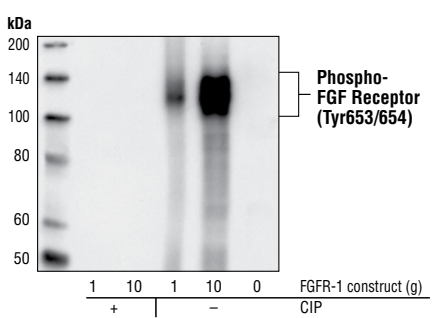
**Background:** Fibroblast growth factors (FGFs) produce mitogenic and angiogenic effects in target cells by signaling through cell surface receptor tyrosine kinases. There are four members of the FGF receptor family: FGFR-1 (flg), FGFR-2 (bek, KGFR), FGFR-3 and FGFR-4. Each receptor contains an extracellular 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 FGFR-1 can be phosphorylated: Tyr463, Tyr583, Tyr585, Tyr653, Tyr654, Tyr730 and Tyr766. Tyrosines 653 and 654 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).

**Specificity/Sensitivity:** Phospho-FGF Receptor (Tyr653/654) (55H2) Mouse mAb detects transfected levels of FGF receptors only when phosphorylated at tyrosines 653/654. The antibody cross-reacts slightly with activated PDGF and insulin/IGF-I receptors.

**Source/Purification:** Monoclonal antibody is produced by immunizing mice with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr653/654 of human FGF receptor-1. The corresponding sequence is identical in FGF receptor-2, -3 and -4.

**Background References:**

- (1) Powers, C.J. et al. (2000) *Endocr. Relat. Cancer* 7, 165–197.
- (2) Reilly, J.F. and Maher, P.A. (2001) *J. Cell Biol.* 275, 7771–7778.
- (3) Mohammadi, M. et al. (1996) *Mol. Cell. Biol.* 16, 977–989.
- (4) Mohammadi, M. et al. (1991) *Mol. Cell. Biol.* 11, 5068–5078.
- (5) Larsson, H. et al. (1999) *J. Biol. Chem.* 274, 25726–25734.



Western blot analysis of extracts from COS cells overexpressing human FGF receptor-1, untreated or calf intestine phosphatase (CIP)-treated, using Phospho-FGF Receptor (Tyr653/654) (55H2) Mouse mAb. Overexpression of human FGF receptor-1 results in constitutive activation of the receptors (courtesy of Dr. Pamela Maher, Scripps Research Institute, California, personal communication). CIP treatment abolishes the reactivity of this antibody to FGF receptor-1.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

\*Species cross-reactivity is determined by Western blot.

**Recommended Antibody Dilutions:**  
Western Blotting 1:1000

**Companion Products:**  
 Phospho-FGF Receptor (Tyr653/654) Antibody #3471  
 Anti-mouse IgG, HRP-linked Antibody #7076  
 Prestained Protein Marker, Broad Range (Premixed Format) #7720  
 Biotinylated Protein Ladder Detection Pack #7727  
 kD20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
 F—Flow cytometry E—ELISA D—DELFIA®  
 Z—zebra fish B—bovine All—all species expected

## Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.