Store at -20°C

Receptor Tyrosine Kinase Antibody Sampler Kit

#42344

1 Kit (8 x 20 µl)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
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Products Included	Product #	Quantity	Mol. Wt.	Isotype
P-Y-1000 MultiMab™ Rabbit mAb mix	8954	20 µl		Rabbit IgG
Met (D1C2) XP® Rabbit mAb	8198	20 µl	140, 170 kDa	Mouse IgG1
EGF Receptor (D38B1) XP® Rabbit mAb	4267	20 µl	175 kDa	Rabbit IgG
PDGF Receptor α (D1E1E) XP® Rabbit mAb	3174	20 µl	190 kDa	Rabbit IgG
PDGF Receptor β (28E1) Rabbit mAb	3169	20 µl	190 kDa	Rabbit IgG
FGF Receptor 1 (D8E4) XP® Rabbit mAb	9740	20 µl	92, 120, 145 kDa	Rabbit IgG
FLT3 (8F2) Rabbit mAb	3462	20 µl	130-160 kDa	Rabbit IgG
HER2/ErbB2 (D8F12) XP® Rabbit mAb	4290	20 µl	185 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

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 antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Description: The Receptor Tyrosine Kinase Antibody Sampler Kit provides the means to detect a broad range of common receptor tyrosine kinases, as well as total phospho-tyrosine activity. The kit provides enough antibody to perform two western blot experiments with each primary antibody.

Background: Tyrosine phosphorylation plays a key role in cellular signaling (1). In cancer studies, unregulated tyrosine kinase activity can drive malignancy and tumor formation by generating inappropriate proliferation and survival signals (2). Antibodies specific for phospho-tyrosine have been invaluable reagents in these studies (3,4).

Met, a tyrosine kinase receptor for hepatocyte growth factor (HGF), is a heterodimer made of α- and β-subunits (5,6). The cytoplasmic region of the β-chain is essential for tyrosine kinase activity. Interaction of Met with HGF results in autophosphorylation at multiple tyrosines (Tyr1003, 1234/1235, 1349) which recruit downstream signaling components, including Gab1, c-Cbl, and PI3 kinase (7-9). Altered Met levels and/or tyrosine kinase activities are found in several types of tumors, including renal, colon, and breast (10,11).

The epidermal growth factor (EGF) receptor is a transmembrane tyrosine kinase that belongs to the HER/ErbB protein family. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling, internalization, and lysosomal degradation (12,13). c-Src mediated phosphorylation of EGF receptor (EGFR) at Tyr845 provides a binding surface for substrate proteins (14-16). The SH2 domain of PLCγ binds at phospho-Tyr992, activating PLCγ-mediated downstream signaling (17). Adaptor protein c-Cbl binds at phospho-Tyr1045, leading to receptor ubiquitination and degradation (18,19). The GRB2 adaptor protein binds activated EGFR at phospho-Tyr1068 (20), while phospho-Tyr1148 and -Tyr1173 provide a docking site for the Shc scaffold protein, playing a role in MAP kinase signaling (13).

Platelet derived growth factor (PDGF) family proteins bind to two closely related receptor tyrosine kinases, PDGF receptor α (PDGFRα) and PDGF receptor β (PDGFRβ) (21). PDGFRα and PDGFRβ can each form heterodimers with EGFR, which is also activated by PDGF (22). Ligand binding induces receptor dimerization and autophosphorylation, followed by binding and activation of signal transduction molecules such as GRB2, Src, GAP, PI3 kinase, PLCγ, and NCK. Signaling pathways initiated by activated PDGF receptors lead to control of cell growth, actin reorganization, migration, and differentiation (23). Tyr751 and Tyr740 of PDGFRβ regulate binding and activation of PI3 kinase (24,25).

Fibroblast growth factors (FGFs) produce mitogenic and angiogenic effects in target cells by signaling through cell surface receptor tyrosine kinases, after ligand binding and dimerization (26,27). Tyr653 and Tyr654 are important for catalytic activity of activated FGFR and are essential for signaling (28). The other phosphorylated tyrosine residues (Tyr463, 583, 585, 730, and 766) may provide docking sites for downstream signaling components such as Crk and PLCγ (29,30).

FMS-related tyrosine kinase 3 (FLT3), a member of the type III receptor tyrosine kinase family, is expressed on early hematopoietic progenitor cells and supports growth and differentiation within the hematopoietic system (31,32). FLT3 is activated after binding with its ligand FL, which results in a cascade of tyrosine autophosphorylation and tyrosine phosphorylation of downstream targets (33). The p85 subunit of PI3 kinase, SHP2, GRB2 and Shc are associated with FLT3 after FL stimulation (34-36). Tyr589/591 may play an important role in regulation of FLT3 tyrosine kinase activity (37).

The ErbB2 (HER2) proto-oncogene encodes a transmembrane, receptor-like glycoprotein with tyrosine kinase activity (38). ErbB2 kinase activity can be activated in the absence of a ligand when overexpressed and through associations with other ErbB family members (39). Phosphorylation at Tyr877 may be involved in regulating ErbB2 activity. Autophosphorylation of ErbB2 at Tyr1248 and Tyr1221/1222 couples ErbB2 to the Ras-Raf-MAP kinase signal transduction pathway (38,40).

Specificity/Sensitivity: Each of the antibodies in the Receptor Tyrosine Kinase Assay Kit recognizes endogenous levels of the specified protein. Phospho-Tyrosine (P-Tyr-1000) MultiMab™ rabbit mAb recognizes a broad range of tyrosine-phosphorylated proteins and peptides. This antibody does not cross-react with proteins or peptides containing phospho-Ser or phospho-Thr residues. EGF Receptor (D38B1) XP® Rabbit mAb does not cross-react with other proteins of the ErbB family. Species cross-reactivity for IHC-P and IF-IC is human only. PDGF Receptor α (D1E1E) XP® Rabbit mAb may cross-react with PDGFRβ at overexpressed levels. Nuclear staining has been observed with this antibody in certain tissues. The specificity of this staining is unknown. PDGF Receptor β (28E1) Rabbit mAb may cross-react with PDGF receptor α at overexpressed levels. FGF Receptor 1 (D8E4) XP® Rabbit mAb may slightly cross-react with overexpressed FGF receptor family members. FLT3 (8F2) Rabbit mAb does not cross-react with related proteins. HER2/ErbB2 (D8F12) XP® Rabbit mAb may slightly cross-react with other overexpressed RTKs.

Source/Purification: MultiMab™ rabbit monoclonal mix antibodies are prepared by combining individual rabbit monoclonal clones in optimized ratios for the approved applications. Each antibody in the mix is carefully selected based on motif recognition and performance in multiple assays. Each mix is engineered to yield the broadest possible coverage of the modification being studied while ensuring a high degree of specificity for the modification or motif. Total monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human Met, the carboxy terminus of human PDGFR α, Ser740 of human FLT3, the amino terminus of human HER2/ErbB2 protein, with a fusion protein containing the cytoplasmic domain of human EGF receptor, with a GST fusion protein containing a carboxy-terminal fragment of human PDGF receptor β, or with a recombinant protein specific to the carboxy terminus of human FGF 1 receptor protein.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.