

# Met Signaling Antibody Sampler Kit

✓ 1 Kit  
(6 x 20 µl)



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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Gab1 (Tyr307) Antibody	3234	20 µl	115 kDa	Rabbit IgG
Gab1 Antibody	3232	20 µl	110 kDa	Rabbit IgG
Phospho-Met (Tyr1003) (13D11) Rabbit mAb	3135	20 µl	145 kDa	Rabbit IgG
Phospho-Met (Tyr1234/1235) (D26) XP® Rabbit mAb	3077	20 µl	145 kDa	Rabbit IgG
Phospho-Met (Tyr1349) (130H2) Rabbit mAb	3133	20 µl	145 kDa	Rabbit IgG
Met (D1C2) XP® Rabbit mAb	8198	20 µl	140 kDa, 170 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Met Signaling Antibody Sampler Kit provides an economical means to investigate Met signaling. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**Background:** Met, a high affinity tyrosine kinase receptor for hepatocyte growth factor (HGF, also known as scatter factor), is a disulfide-linked heterodimer made of 45 kDa  $\alpha$ - and 145 kDa  $\beta$ -subunits (1,2). The  $\alpha$ -subunit and the amino-terminal region of the  $\beta$ -subunit form the extracellular domain. The remainder of the  $\beta$ -chain spans the plasma membrane and contains a cytoplasmic region with tyrosine kinase activity. Interaction of Met with HGF results in autophosphorylation at multiple tyrosines, which recruit several downstream signaling components, including Gab1, c-Cbl and PI3 kinase (3). These fundamental events are important for all of the biological functions involving Met kinase activity. Addition of a phosphate at cytoplasmic Tyr1003 is essential for ubiquitination and Met protein degradation (4). Phosphorylation of Tyr1234/1235 in the Met kinase domain is critical to kinase activation. Phosphorylation of Tyr1349 in the Met cytoplasmic domain provides a direct binding site for Gab1 (5). Altered Met levels and/or tyrosine kinase activities are found in several types of tumors, including renal, colon and breast cancers. Thus, Met is an attractive cancer therapeutic and diagnostic target (6).

**Specificity/Sensitivity:** Each antibody in the Met Signaling Antibody Sampler Kit recognizes endogenous levels of its specific target and does not cross-react with other family members unless otherwise indicated. Phospho-Met (Tyr1234/1235) (D26) Rabbit mAb may cross-react with overexpressed tyrosine phosphorylated Src proteins in Western blot. Phospho-Met (Tyr1349) (130H2) Rabbit mAb may cross-react with other activated protein tyrosine kinases. Phospho-Met (Tyr1003) (13D11) Rabbit mAb may cross-react with other activated protein tyrosine kinases. Phospho-Gab1 (Tyr307) Antibody cross-reacts with phosphorylated Gab2 and potentially with phosphorylated Gab3.

**Source/Purification:** Monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides or peptides corresponding to residues surrounding: Tyr1003, Tyr1234/1235, Tyr1349, or near the carboxy terminus of human Met protein. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide or peptide corresponding to residues surrounding Tyr472 and Tyr307 of human Gab1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

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

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

Please visit [www.cellsignal.com](http://www.cellsignal.com) for validation data and a complete listing of recommended companion products.

U.S. Patent No. 5,675,063  
Tween®20 is a registered trademark of ICI Americas, Inc.

**Background References:**

- (1) Weidner, K.M. et al. (1993) *Mol Immunol* 30, 1003–11.
- (2) Park, M. et al. (1986) *Cell* 45, 895–904.
- (3) Bardelli, A. et al. (1997) *Oncogene* 15, 3103–11.
- (4) Taher, T.E. et al. (2002) *J Immunol* 169, 3793–800.
- (5) Schaeper, U. et al. (2000) *J Cell Biol* 149, 1419–32.
- (6) Traxler, P. et al. (2001) *Med Res Rev* 21, 499–512.

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

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 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% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween®20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) 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 markers (#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.