Met Signaling Antibody Sampler Kit

#3019 Store at –20°C

☑ 1 Kit
(6 x 40 µl)

Cell Signaling TECHNOLOGY®

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Gab1 (Tyr307) Antibody	3234	40 µl	115 kDa	Rabbit IgG
Gab1 Antibody	3232	40 µl	110 kDa	Rabbit IgG
Phospho-Met (Tyr1003) (13D11) Rabbit mAb	3135	40 µl	145 kDa	Rabbit IgG
Phospho-Met (Tyr1234/1235) (D26) XP™ Rabbit mAb	3077	40 µl	145 kDa	Rabbit IgG
Phospho-Met (Tyr1349) (130H2) Rabbit mAb	3133	40 µl	145 kDa	Rabbit IgG
Met (25H2) Mouse mAb	3127	40 µl	145 kDa, 170 kDa	Mouse IgG1
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

See 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 contains primary and secondary antibodies to perform four Western mini-blots with each 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 α- and 145 kDa β-subunits (1,2). The α-subunit and the amino-terminal region of the β-subunit form the extracellular domain. The remainder of the β-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) XP™ 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 (KLH-coupled) corresponding to residues surrounding: Tyr1003, Tyr1234/1235, Tyr1349, or Tyr1234 of human Met. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide or peptide (KLH-coupled) corresponding to residues surrounding Tyr472 and Tyr307 of human Gab1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

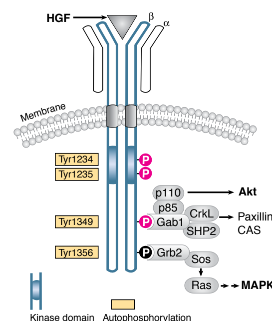
Background References:

(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.

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 for a complete listing of recommended companion products.



Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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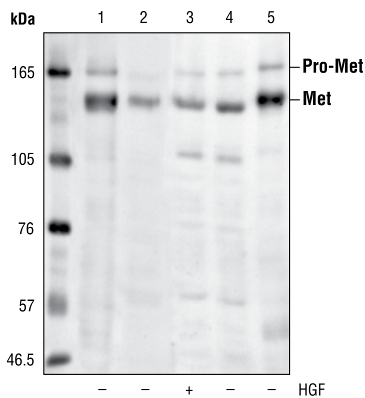
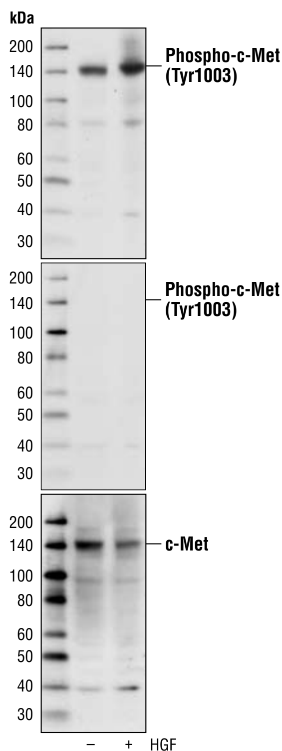
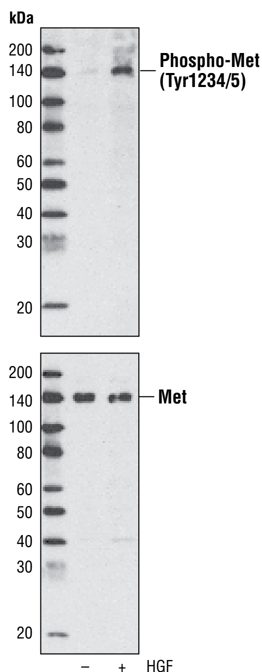


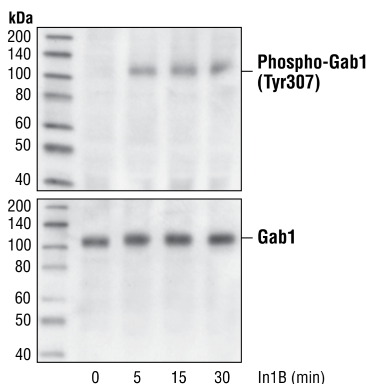
Western blot analysis of extracts from various cell lines: mIMCD3 (lane 1), Vero (lane 2), 293 (HGF stimulated; lane 3) 293 (starved; lane 4) and C6 (lane 5) using ***Met (25H2) Mouse mAb #3127.***



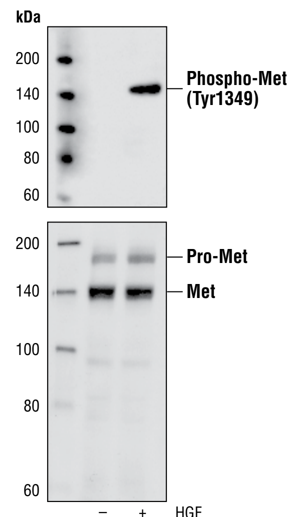
Western blot analysis of extracts from A431 cells, untreated or HGF-treated, using ***Phospho-Met (Tyr1003) (13D11) Rabbit mAb #3135*** *(upper and middle) and* ***Met (25H2) Mouse mAb #3127*** *(lower). The middle blot was treated with CIP phosphatase before antibody probing.*



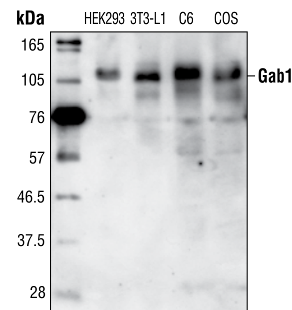
Western blot analysis of cell extracts from HeLa cells, untreated or stimulated with HGF, using ***Phospho-Met (Tyr1234/1235) (D26) XP™ Rabbit mAb #3077*** *(upper) and* ***Met (25H2) Mouse mAb #3127*** *(lower).*



Western blot analysis of extracts from HEK293 cells, untreated or treated with In1B for indicated times, using ***Phospho-Gab1 (Tyr307) Antibody #3234*** *(upper) or* ***Gab1 Antibody #3232*** *(lower).*



Western blot analysis of cell lysates of H4IIE cells, untreated or treated with HGF, using ***Phospho-Met (Tyr1349) (130H2) Rabbit mAb #3133*** *(upper), or* ***Met (25H2) Mouse mAb #3127*** *(lower).*



Western blot analysis of extracts from various cells lines using ***Gab1 Antibody #3232.***

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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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.

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