Store at -20°C

Matrix Remodeling Antibody Sampler Kit

#73959

☑ 1 Kit
(9 x 20 µl)

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

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

New 10/17

Entrez-Gene ID #4323, 4313, 4314, 4316, 4318, 7076, 7077, 7078
UniProt ID #P50281, P08253, P08254, P09237, P14780, P01033, P16035, P35625

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
MMP-9 (D6O3H) XP® Rabbit mAb	13667	20 µl	84, 92 kDa	Rabbit IgG
MMP-2 (D2O4T) Rabbit mAb	87809	20 µl	64,72 kDa	Rabbit IgG
MT1-MMP (D1E4) Rabbit mAb	13130	20 µl	50, 62 kDa	Rabbit IgG
MMP3 (D7F5B) Rabbit mAb	14351	20 µl	60 kDa	Rabbit IgG
TIMP1 (D10E6) Rabbit mAb	8946	20 µl	26 kDa	Rabbit IgG
TIMP2 (D18B7) Rabbit mAb	5738	20 µl	22 kDa	Rabbit IgG
TIMP3 (D74B10) Rabbit mAb	5673	20 µl	20, 25 kDa	Rabbit IgG
MMP-7 Antibody	71031	20 µl	28 kDa	Rabbit
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.

Description: The Matrix Remodeling Antibody Sampler Kit provides an economical means of detecting different MMPs and TIMPs using the specific corresponding antibodies. The kit contains enough antibody to perform at least two western blot experiments with each primary antibody.

Background: Matrix remodeling is mainly controlled by MMPs and TIMPs. The matrix metalloproteinase (MMP) family of proteases are a group of zinc-dependent enzymes that target extracellular proteins, including growth factors, cell surface receptors, adhesion molecules, matrix structural proteins, and other proteases (1, 2). Among the family members, MMP-2, MMP-3, MMP-7, MMP-9, and MMP14 (MT1-MMP) have been characterized as important factors for normal tissue remodeling during embryonic development, wound healing, tumor invasion, angiogenesis, carcinogenesis, and apoptosis (3). MMP activity is regulated by mechanisms of both transcriptional control and post translational protein processing. Once synthesized, MMPs exist as latent proenzymes. Maximum MMP activity requires proteolytic cleavage to generate active MMPs by releasing the inhibitory propeptide domain from the full-length protein (4). MMP activity can be inhibited through its binding to endogenously expressed TIMPs. TIMPs are members of the family of tissue inhibitors of matrix metalloproteinases that include TIMP1, TIMP2, TIMP3, and TIMP4. The main function of TIMPs is their inhibitory effect on MMPs. TIMPs irreversibly inactivate MMPs by direct binding MMPs and chelating their zinc cofactor at the catalytic site to inhibit the proteinase function (5,6).

Specificity/Sensitivity: Each antibody in the Matrix Remodeling Antibody Sampler Kit detects endogenous levels of its target protein.

Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with synthetic peptides corresponding to Pro117 of human MMP2, Ser417 of human MMP3, Phe542 of human MMP9, Met293 of MT1-MMP, Ala134 of human TIMP1, Thr135 of human TIMP2, and Lys53 of human TIMP3. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to Phe98 of human MMP-7. 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, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

Background References:

(1) Kessenbrock, K. et al. (2010) *Cell* 141, 52-67.

(2) McCawley, L.J. and Matrisian, L.M. (2001) *Curr Opin Cell Biol* 13, 534-40.

(3) Page-McCaw, A. et al. (2007) *Nat Rev Mol Cell Biol* 8, 221-33.

(4) Hadler-Olsen, E. et al. (2011) *FEBS J* 278, 28-45.

(5) Nagase, H. et al. (2006) *Cardiovasc Res* 69, 562-73.

(6) Visse, R. and Nagase, H. (2003) *Circ Res* 92, 827-39.

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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.

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 Detection Pack:** (#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.