Store at -20°C

#25620

Human Reactive Inflammasome Antibody Sampler Kit II

1 Kit
(8 x 20 µl)

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

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

New 03/19

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
NLRP3 (D4D8T) Rabbit mAb	15101	20 µl	110 kDa	Rabbit IgG
AIM2 (D5X7K) Rabbit mAb	12948	20 µl	40 kDa	Rabbit IgG
NLRC4 (D5Y8E) Rabbit mAb	12421	20 µl	110 kDa	Rabbit IgG
Caspase-1 (D7F10) Rabbit mAb	3866	20 µl	20, 48 kDa	Rabbit IgG
ASC/TMS1 (E1E3I) Rabbit mAb	13833	20 µl	15, 19, 22 kDa	Rabbit IgG
Cleaved Caspase-1 (Asp297) (D57A2) Rabbit mAb	4199	20 µl	20, 22 kDa	Rabbit IgG
Cleaved-IL-1β (Asp116) (D3A3Z) Rabbit mAb	83186	20 µl	17 kDa	Rabbit IgG
IL-1β (D3U3E) Rabbit mAb	12703	20 µl	17, 31 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.

Description: The Human Reactive Inflammasome Antibody Sampler Kit II provides an economical means of detecting multiple inflammasome components. The kit contains enough primary antibodies to perform at least two western blot experiments.

Background: The innate immune system works as the first line of defense in protection from pathogenic microbes and host-derived signals of cellular distress. One way in which these "danger" signals trigger inflammation is through activation of inflammasomes, which are multiprotein complexes that assemble in the cytosol after exposure to pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) and result in the activation of caspase-1 and subsequent cleavage of proinflammatory cytokines IL-1β and IL-18 (Reviewd in 1-6). Inflammasome complexes typically consist of a cytosolic pattern recognition receptor (PRR; a nucleotide-binding domain and leucine-rich-repeat [NLR] or AIM2-like receptor [ALR] family member), an adaptor protein (ASC/TMS1), and pro-caspase-1. A number of distinct inflammasome complexes have been identified, each with a unique PRR and activation triggers. The best characterized is the NLRP3 complex, which contains NLRP3, ASC/TMS1, and pro-caspase-1. The NLRP3 inflammasome is activated in a two-step process. First, NF-κB signaling is induced through PAMP- or DAMP-mediated activation of TLR4 or TNFR, resulting in increased expression of NLRP3, pro-IL-1β, and pro-IL-18 (priming step, signal 1). Next, indirect activation of NLRP3 occurs by a multitude of signals (whole pathogens, PAMPs/DAMPs, potassium efflux, lysosomal-damaging environmental factors [uric acid, silica, alum] and endogenous factors [amyloid-β, cholesterol crystals], and mitochondrial damage), leading to complex assembly and activation of caspase-1 (signal 2). The complex inflammasome structure is built via domain interactions among the protein components. Other inflammasomes are activated by more direct means: double-stranded DNA activates the AIM2 complex, anthrax toxin activates NLRP1, and bacterial flagellin activates NLRC4. Activated caspase-1 induces secretion of proinflammatory cytokines IL-1β and -18, but also regulates metabolic enzyme expression, phagosome maturation, vasodilation, and pyroptosis, an inflammatory programmed cell death. Inflammasome signaling contributes to the onset of a number of diseases, including atherosclerosis, type II diabetes, Alzheimer's disease, and autoimmune disorders.

Specificity/Sensitivity: Each antibody in the Human Reactive Inflammasome Antibody Sampler Kit II detects endogenous levels of its target protein. AIM2 (D5X7K) Rabbit mAb detects a 22 kDa band of unknown origin in some cell lines. Caspase-1 (D7F10) Rabbit mAb detects endogenous levels of full-length human Caspase-1; the activated p20 subunit was detected by overexpression. ASC/TMS1 (E1E3I) Rabbit mAb can detect three known isoforms of ASC/TMS1. Cleaved Caspase-1 (Asp297) (D57A2) Rabbit mAb detects endogenous levels of the p20 subunit of human caspase-1 only upon cleavage at Asp297. Cleaved-IL-1β (Asp116) (D3A3Z) Rabbit mAb recognizes endogenous levels of mature IL-1β protein only when cleaved at Asp116. IL-1β (D3U3E) Rabbit mAb is not able to detect endogenous levels of mature IL-1β. It can detect up to 100 pg of recombinant mature IL-1β.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with recombinant human IL-1β protein or with synthetic peptides corresponding to residues adjacent to Asp297 human caspase-1, residues adjacent to Asp116 of human IL-1β, residues surrounding Ala306 of mouse NLRP3, Lys93 of human AIM2, Leu942 of human NLRC4, and the carboxy terminus of human ASC/TMS1 isoform 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.*

Background References:

(1) Broz, P. and Dixit, V.M. (2016) *Nat Rev Immunol* 16, 407-20.

(2) Guo, H. et al. (2015) *Nat Med* 21, 677-87.

(3) Jo, E.K. et al. (2016) *Cell Mol Immunol* 13, 148-59.

(4) Rathinam, V.A. and Fitzgerald, K.A. (2016) *Cell* 165, 792-800.

(5) Shao, B.Z. et al. (2015) *Front Pharmacol* 6, 262.

(6) Schroder, K. and Tschopp, J. (2010) *Cell* 140, 821-32.

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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 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.

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