#4769 Store at –20°C

IRAK Isoform Antibody Sampler Kit

☑ 1 Kit
(4 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
IRAK1 (D51G7) Rabbit mAb	4504	20 µl	78, 105 kDa	Rabbit IgG
IRAK2 Antibody	4367	20 µl	62 kDa	Rabbit IgG
IRAK-M Antibody	4369	20 µl	68 kDa	Rabbit IgG
IRAK4 Antibody	4363	20 µl	55 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 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 validation data and a complete listing of recommended companion products.

Description: The IRAK Isoform Antibody Sampler Kit provides an economical means to examine total protein levels of the four Interleukin-1 Receptor Associated Kinase family members: IRAK1, IRAK2, IRAK3/IRAK-M, and IRAK4.

Background: Interleukin-1 (IL-1) receptor-associated kinase (IRAK) is a serine/threonine-specific kinase that can be coprecipitated in an IL-1-inducible manner with the IL-1 receptor (1). The mammalian family of IRAK molecules contains four members (IRAK1, IRAK2, IRAK3/IRAK-M and IRAK4). The binding of IL-1 to IL-1 receptor type I (IL-1RI) initiates the formation of a complex that includes IL-1RI, AcP, MyD88 and IRAKs (2). IRAK undergoes autophosphorylation shortly after IL-1 stimulation. The subsequent events involve IRAK dissociation from the IL-1RI complex, its ubiquitination and its association with two membrane-bound proteins: TAB2 and TRAF6. The resulting IRAK-TRAF6-TAB2 complex is then released into the cytoplasm and activates protein kinase cascades, which include TAK1, IKKs and the stress-activated kinases (3).

Upon IL-1R/TLR (Toll-Like Receptor) ligation, IRAK1 and IRAK4 are rapidly recruited to the receptor by the adaptor MyD88 (4). IRAK1 is phosphorylated by IRAK4 at Thr209 and Thr387 (5), followed by sequential autohyperphosphorylation in various domains. Unlike IRAK1 and IRAK4, IRAK2 and IRAK-M do not have significant kinase activity although they can still activate NF-κB when overexpressed (6,7). Antisense oligonucleotide depletion of IRAK2 can inhibit IL-1 mediated NF-κB activation (8). Expression of IRAK-M is more restricted compared to other family members with highest levels of expression occurring in monocytes/macrophages (6). Studies from IRAK-M knockout mice suggest that it may play a role as a negative regulator of TLR signaling and innate immune responses by preventing the dissociation of IRAK1 and IRAK4 from MyD88 and the subsequent formation of its complex with TRAF6 (9).

Specificity/Sensitivity: Each antibody in the IRAK Isoform Antibody Sampler Kit detects endogenous levels of its indicated target. Cross-reactivity has not been detected with other family members at endogenous levels.

Source/Purification: Antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy-terminus of murine IRAK1, human IRAK2, human IRAK-M, and surrounding Lys41 of human IRAK4.

Background References:

(1) Dinarello, C.A. (1996) *Blood* 87, 2095–2147.
(2) Takaesu, G. et al. (2001) *Mol. Cell. Biol.* 21, 2475–2484.
(3) Janssens, S. and Beyaert, R. (2003) *Mol. Cell* 11, 293–302.
(4) Gottipati, S. et al. (2008) *Cell Signal* 20, 269–76.
(5) Kollewe, C. et al. (2004) *J Biol Chem* 279, 5227–36.
(6) Wesche, H. et al. (1999) *J Biol Chem* 274, 19403–10.
(7) Muzio, M. et al. (1997) *Science* 278, 1612–5.
(8) Guo, F. et al. (1999) *Inflammation* 23, 535–43.
(9) Kobayashi, K. et al. (2002) *Cell* 110, 191–202.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **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% sequence homology.

#4769

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.