

#4769 Store at -20°C

IRAK Isoform Antibody Sampler Kit

✓ 1 Kit
(4 x 40 µl)



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rev. 11/01/10

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
IRAK1 (D51G7) XP™ Rabbit mAb	4504	40 µl	78, 105 kDa	Rabbit IgG
IRAK2 Antibody	4367	40 µl	62 kDa	Rabbit IgG
IRAK-M Antibody	4369	40 µl	68 kDa	Rabbit IgG
IRAK4 Antibody	4363	40 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.

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.

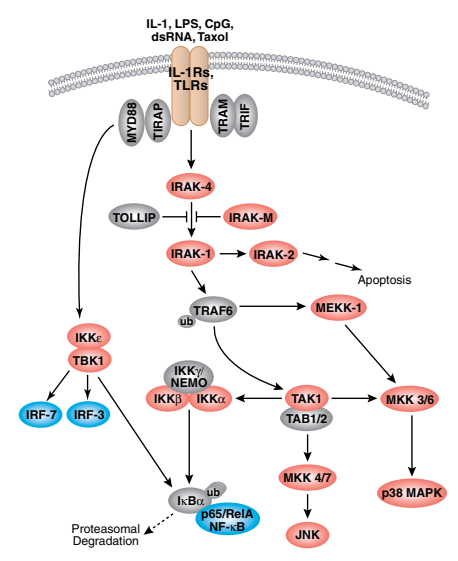
Source/Purification: Antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy-terminus of murine IRAK1, human IRAK2, human IRAK-M, and surrounding Lys41 of human IRAK4.

- 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.

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.

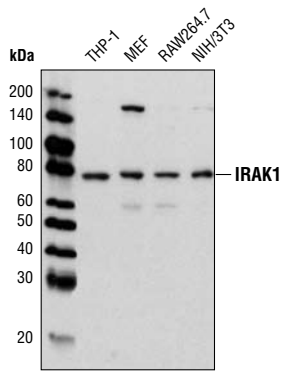


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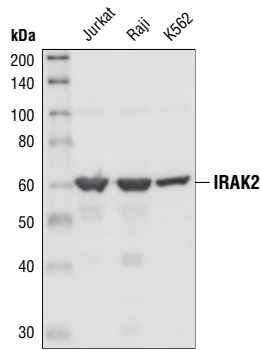
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA E-P—ELISA Peptide

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

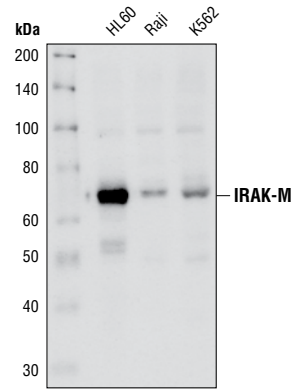
Dg—Dog Pg—Pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.



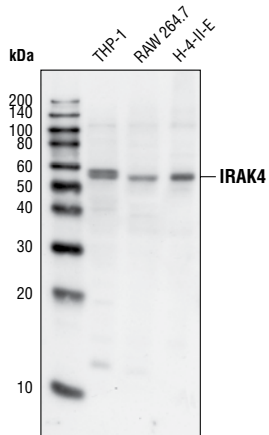
Western blot analysis of extracts from THP-1, mouse embryonic fibroblast (MEF), RAW264.7 and NIH/3T3 cells using **IRAK1 (D51G7) XP™ Rabbit mAb #4504**.



Western blot analysis of extracts from Jurkat, Raji and K562 cell lines, using **IRAK2 Antibody #4367**.



Western blot analysis of extracts from HL-60, Raji and K562 cell lines, using **IRAK-M Antibody #4369**.



Western blot analysis of extracts from THP-1 (human), RAW 264.7 (mouse), and H-4-II-E (rat) cell lines, using **IRAK4 Antibody #4363**.

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 cm²) 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.