

#4537 Store at -20°C

Phospho-TAK1 (Thr184) Antibody



✓ 100 µl
(10 Western mini-blot)

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rev. 01/10/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

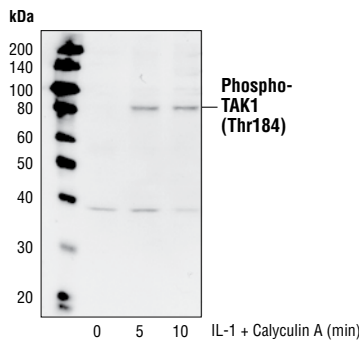
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, (M, R, C, B, X, Z)	82 kDa	Rabbit

Background: TAK1 is a mitogen-activated protein kinase kinase kinase that can be activated by TGFβ, bone morphogenetic protein and other cytokines including IL-1 (1,2). *In vivo* activation of TAK1 requires association with TAK1 binding protein 1 (TAB1), which triggers phosphorylation of TAK1 (3,4). Another adaptor protein, TAB2, links TAK1 with TRAF6 and mediates TAK1 activation upon IL-1 stimulation (5). Once activated, TAK1 phosphorylates MAPK kinases MKK4 and MKK3/6, which activate p38 MAPK and JNK, respectively. In addition, TAK1 activates the NF-κB pathway by interacting with TRAF6 and phosphorylating the NF-κB inducing kinase (NIK) (2).

TAK1 activation requires multiple phosphorylations in its activation loop. Mutation of Thr187 and Thr184, residues located in the activation loop of TAK1, impairs phosphorylation of both TAK1 and TAB1 and reduces the kinase activity of TAK1, suggesting that autophosphorylation of these residues is necessary for TAK1 activation (4).

Specificity/Sensitivity: Phospho-TAK 1(Thr184) Antibody detects endogenous levels of TAK1 only when phosphorylated at Thr184.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr184 of human TAK1. Antibodies are purified by protein A and affinity chromatography.



Western blot analysis of extracts from 293 IL-1R cells treated with IL-1β (10 ng/ml) and Calyculin A (50 nM) for the indicated times using Phospho-TAK1 (Thr184) Antibody.

Background References:

- (1) Yamaguchi, K. et al. (1995) *Science* 270, 2008–2011.
- (2) Ninomiya-Tsuji, J. et al. (1999) *Nature* 398, 252–256.
- (3) Shibuya, H. et al. (1996) *Science* 272, 1179–1182.
- (4) Sakurai, H. et al. (2000) *FEBS Lett.* 474, 141–145.
- (5) Takaesu, G. et al. (2000) *Mol. Cell* 4, 649–658.

Entrez-Gene ID #6885
Swiss-Prot Acc. #043318

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 antibody.*

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:
Western Blotting 1:1000

Companion Products:

- Phospho-TAK1 (Thr187) Antibody #4536
- Phospho-TAK1 (Thr184/187) Antibody #4531
- Phospho-TAK1 (Thr184/187) (90C7) Rabbit mAb #4508
- TAK1 Antibody #4505
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM
Z—zebra fish B—bovine All—all species expected

Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 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)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) 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 marker (#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.