Store at -80°C

#7551

IRAK4 Kinase

☑ 5 µg

Cell Signaling TECHNOLOGY®

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REV. 12/06/05

Description: Purified recombinant human IRAK4 kinase, supplied as a GST fusion protein.

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, 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).

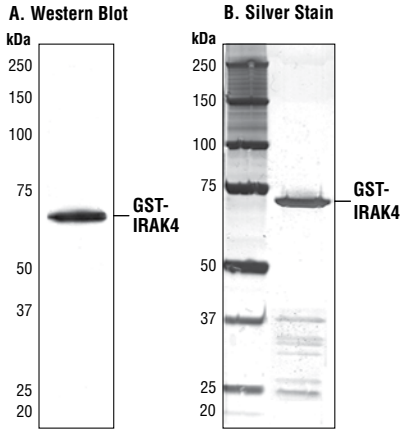

Figure 1. The purity of the IRAK4 fusion protein was analyzed using SDS/PAGE followed by anti-IRAK4 Western blot (A) or Silver stain (B).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human IRAK4 (Ala104-Ser460) (GenBank Accession No. AF445802.1) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography purification using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-IRAK4 fusion protein is 70 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. IRAK4 kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure IRAK4 activity using HTScan™ IRAK4 Kinase Assay Kit #7552 [Fig.3].

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.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ IRAK4 Kinase Assay Kit #7552

Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide #1344

Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

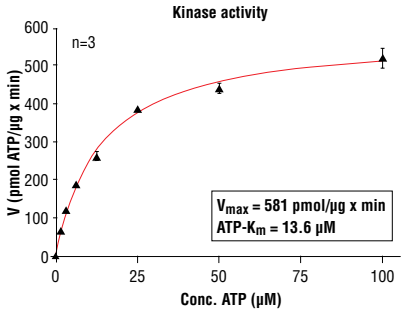

Figure 2. IRAK4 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 uM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 ug/50 ul PEG20,000, Substrate: Histone H2B, 5 ug/50 ul, Recombinant IRAK4: 20 ng/50 ul.

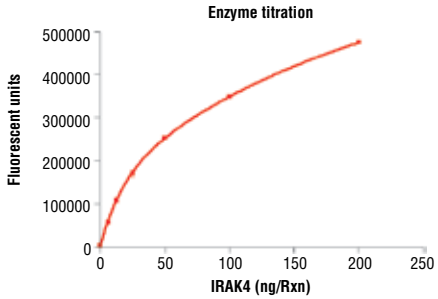

Figure 3. Dose dependence curve of IRAK4 kinase activity: DELFIA® data generated using Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141 to detect phosphorylation of substrate peptide (#1344) by IRAK4 kinase. In a 50 µl reaction, increasing amounts of IRAK4 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for IRAK4 Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141
5. Kinase Buffer (10X) #9802
6. ATP (10 mM) #9804
7. Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide #1344
8. DELFIA® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
9. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

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B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng IRAK4 Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    Please note: This protocol was validated using a Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Biotinylated Peptide and Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com