

IRAK4, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # I12-10G

Lot # W234-1

Product Description

Recombinant full-length human IRAK4 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC013316](#).

Gene Aliases

IPD1, REN64, NY-REN-64

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

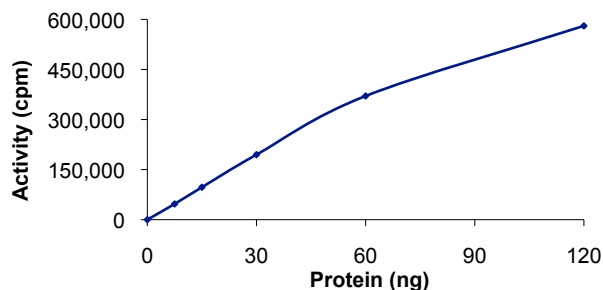
Scientific Background

Interleukin-1 receptor-associated kinase 4 (IRAK4) is an important mediator in the signal transduction of Toll-like receptor (TLR) and IL1R family members (1). IRAK4 is involved in the Toll-like receptor signaling pathway leading to Apoptosis. IRAK4 has molecular functions like protein binding, ATP binding, kinase activity and magnesium ion binding. Toll/IL-1 receptor family members like IRAK4 are central components of host defense mechanisms in a variety of species (2). One well conserved element in their signal transduction is the Ser/Thr kinase activity which couple early signaling events in a receptor complex at the plasma membrane to larger signalosomes in the cytosol.

References

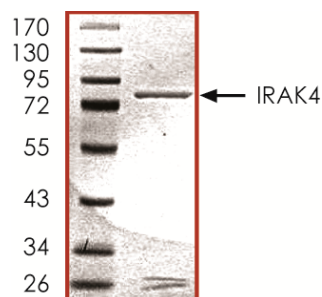
1. Li, S. et al: IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. Proc. Natl. Acad. Sci. USA. 2002; 99:5567-72.
2. Suzuki, N. et al: IRAK-4 as the central TIR signaling mediator in innate immunity. Trends Immunol. 2002; 23:503-6

Specific Activity



The specific activity of IRAK4 was determined to be **393 nmol /min/mg** as per activity assay protocol.

Purity



The purity of IRAK4 was determined to be **>85%** by densitometry, approx. MW **81kDa**.

IRAK4, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	I12-10G
Specific Activity	393 nmol/min/mg
Specific Lot Number	W234-1
Purity	>85%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: I12-10G)

Active IRAK4 (0.1 µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active IRAK4 for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H₂O.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100 µl [³³P]-ATP (1mCi/100 µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200 µl aliquots at -20°C.

Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active IRAK4, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 µl:
- Component 1.** 10 µl of diluted Active IRAK4 (Catalog #I12-10G)
 - Component 2.** 5 µl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5 µl distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25 µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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