SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
I12-10G-05	5 µg
I12-10G-10	10 µg

IRAK4, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # I12-10G
Lot # J438-2

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.

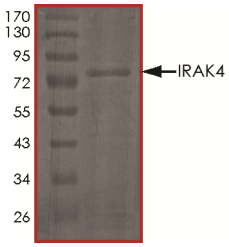
References

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 **400 nmol /min/mg** as per activity assay protocol.

Purity



The purity of IRAK4 was determined to be **>90%** by densitometry, approx. MW **81kDa**.

IRAK4, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	I12-10G
Specific Activity	400 nmol/min/mg
Lot #	J438-2
Purity	>90%
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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: I12-10G)

Active IRAK4 (0.1μg/μl) diluted with Kinase Dilution Buffer (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 (Catalog #: K23-09)

Kinase Assay Buffer (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H_2O.

Kinase Assay Buffer (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer (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 (Catalog #: K01-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: M42-51N)

Myelin basic protein (MBP) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active IRAK4, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active IRAK4 (Catalog #I12-10G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #M42-51N)
- **Component 3.** 5μl distilled H_2O (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 H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl 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 H_2O) with constant gentle stirring. It is recommend 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 [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-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 ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com