

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human Jak1 protein				
	Met	6-His tag	Sumo-tag (mutated, uncleavable)	3c Protease site	Human JAK1 (Ala561-Lys1154) Accession # P23458.2
N-terminal Sequence Analysis	Protein identity confirmed by mass spectrometry.				
Predicted Molecular Mass	81 kDa				

SPECIFICATIONS

SDS-PAGE	75-89 kDa, under reducing conditions
Activity	Measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a peptide substrate. The specific activity is >110 pmol/min/μg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 μg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 μm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials	- Assay Buffer: 50 mM Tris, 20 mM MgCl₂, 5 mM MnCl₂, 0.1 mg/mL BSA, pH 7.5 - Recombinant Human JAK1 kinase domain His-tag (rhJAK1) (Catalog # 11738-J1) - IRS-1 (TYR608) peptide, 1 mg/mL stock in deionized water - Adenosine triphosphate (ATP), 10 mM stock in deionized water - ADP-Glo™ Kinase Assay (Promega) - White 96-well Plate - Plate Reader with Luminescence Read Capability
Assay	- Dilute rhJAK1 to 5 μg/mL in Assay Buffer. - Prepare Substrate Mixture containing 200 μM ATP and 0.4 mg/mL IRS-1 (TYR608) peptide in Assay Buffer. - Combine equal volumes of 5 μg/mL rhJAK1 and Substrate Mixture. Create a Substrate Control by replacing enzyme with Assay Buffer. - Incubate at room temperature for 40 minutes in the dark. - After incubation, transfer 10 μL of each reaction to wells of a white plate. - Terminate the reaction and deplete the remaining ATP by adding 10 μL of ADP-Glo Reagent (supplied in kit) to all wells. - Incubate at room temperature for 40 minutes in the dark. - Add 20 μL Kinase Detection Reagent (supplied in kit) to all wells. - Incubate at room temperature for 30 minutes in the dark. - Read plate in Luminescence endpoint mode. - Calculate specific activity: $\text{Specific Activity (pmol/min/μg)} = \frac{\text{Adjusted Luminescence* (RLU)} \times \text{Conversion Factor** (pmol/RLU)}}{\text{Incubation time (min)} \times \text{amount of enzyme (μg)}}$ *Adjusted for Substrate Control **Derived from ADP-Glo™ Kinase Assay Kit protocol (Promega)

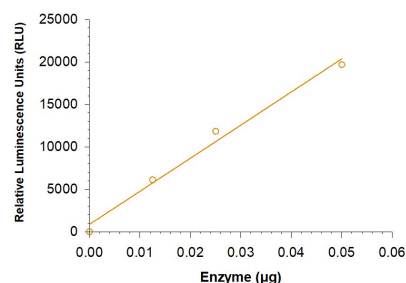
Final Assay Conditions	Per Reaction: - rhJAK1: 2.5 μg/mL - ATP: 100 μM - IRS-1 (Tyr608) peptide: 0.2 mg/mL
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PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. 6 months from date of receipt, -20 to -70 °C as supplied. 3 months, -20 to -70 °C under sterile conditions after opening.

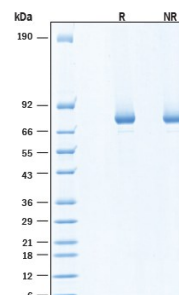
DATA

Enzyme Activity



Recombinant Human JAK1 kinase domain His-tag Enzyme Activity. Recombinant Human JAK1 kinase domain His-tag (Catalog # 11738-J1) is measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a peptide substrate.

SDS-PAGE



Recombinant Human JAK1 kinase domain His-tag SDS-PAGE. 2 µg/lane of Recombinant Human JAK1 kinase domain His-tag (Catalog # 11738-J1) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 75-89 kDa, under reducing conditions.

BACKGROUND

Janus Kinase 1 (JAK1) is one of four members of the JAK, or "just another kinase", family of intracellular, non-receptor tyrosine kinases (1) that noncovalently associate with Type I and Type II cytokine receptor cytoplasmic regions to mediate receptor tyrosine and subsequent STAT protein phosphorylation. JAK1 is broadly expressed in many cell types and contains several conserved JAK homology (JH) domains that compose an N-terminal FERM domain followed by a Src homology (SH) domain that associate and mediate interaction with the cytokine receptors (2,3) and a pseudokinase domain with no catalytic activity that regulates the activity of the catalytic kinase domain present at the C-terminus (4). JAK/STAT signaling is activated by over 50 cytokines that bind to the major cytokine receptors prompting auto-activation of JAKs by transphosphorylation and subsequent phosphorylation of the receptors to promote STAT binding (1,4). Once STAT proteins are bound, JAKs phosphorylate STAT causing dissociation, dimerization, and translocation to the nucleus where they function as transcription factors and play a role in the control of hematopoiesis, the immune response, embryogenesis, inflammation, and cancer (5-8). In addition, JAK1 activating mutations have been directly reported in Castleman's disease (9) and T-cell malignancies such as activating T-ALL, Sezary Syndrome, and T-cell prolymphocytic leukemia (10-12). There is interest in development of JAK inhibitors as a therapeutic target to prevent phosphorylation of their substrates and downstream signaling pathways. Prior clinical studies targeting JAK1 have been focused on rheumatoid arthritis, myelofibrosis, and dermatology-related conditions such as psoriasis (1,13-15).

References:

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