Recombinant Human IL-4I1

Catalog Number: 5684-AO

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human IL-4I1 protein Gln22-His567, with a C-terminal 6-His tag Accession # Q96RQ9
N-terminal Sequence Analysis	No results obtained: Gln22 predicted
Structure / Form	Monomer
Predicted Molecular Mass	61 kDa

SPECIFICATIONS

SDS-PAGE	70-75 kDa, reducing conditions
Activity	Measured by its ability to oxidize phenylalanine in a horseradish peroxidase coupled assay. The specific activity is >150 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, 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 and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Sodium Phosphate, pH 7.0
- Recombinant Human IL-4I1 (rhIL-4I1) (Catalog # 5684-AO)
- Coupling Agent: Horseradish Peroxidase (HRP) (250-330 U/mg) (Sigma, Catalog # P8375), 250 units/mL stock in 0.1 M Sodium Phosphate, pH 8.0
- Substrate Component 1: Phenylalanine (Sigma, Catalog # P2126), 100 mM stock in diH_2O
- Substrate Component 2: Amplex Ultra Red (AUR) (Molecular Probes, Catalog # A36006), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhIL-4I1 to 2 ng/µL in Assay Buffer.
2. Prepare the Substrate mixture including 2 mM Phenylalanine, 2 units/mL HRP and 100 µM AUR in Assay Buffer.
3. Load in a black well plate 50 µL of 2 ng/µL of rhIL-4I1, and start the reaction by adding 50 µL of the Substrate mixture (step 2). Include a Substrate Blank containing 50 µL of the Assay Buffer and 50 µL of the Substrate mixture.
4. Read at excitation and emission wavelengths of 544 nm and 590 nm (top read), respectively in kinetic mode for 5 minutes. **Note:** A wavelength cutoff of 570 nm should be manually specified.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using a fluorescent standard prepared by incubating 50 µM AUR, 1 unit/mL HRP, 1 mM Phenylalanine, and a range of Hydrogen Peroxide (0-500 pmol) (Sigma, Catalog # H1009) in Assay Buffer at room temperature. Use the resulting amounts of oxidized AUR to determine the conversion factor.

Final Assay Conditions

Per Well:

- rhIL-4I1: 0.1 µg
- Phenylalanine: 1 mM
- HRP: 1 unit/mL
- AUR: 50 µM

Caution: The rhIL-4I1 enzymatic reaction may significantly be interfered with organic buffer such as MES.

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

DATA

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Recombinant Human IL-4I1

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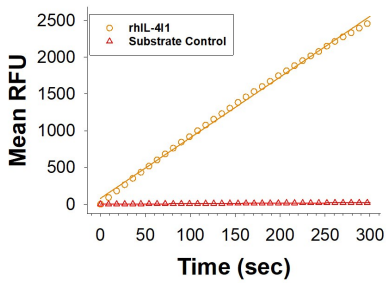
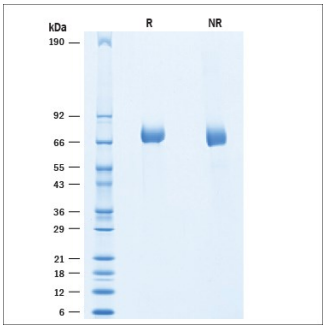
Enzyme Activity



Enzyme Activity of Human IL-4I1 Protein Recombinant Human IL-4I1 Protein (Catalog # 5684-AO) is measured by its ability to oxidize phenylalanine in a horseradish peroxidase coupled assay.

SDS-PAGE



SDS-PAGE of Human IL-4I1 Protein 2 µg/lane of Recombinant Human IL-4I1 Protein (Catalog # 5684-AO) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at ~71 under reducing conditions.

BACKGROUND

Interleukin 4 induced protein 1 (IL-4I1), also known as protein FIG-1 and L-amino acid oxidase, is encoded by a B-cell IL-4-inducible gene, FIG1, and is highly expressed in primary metastinal B-cell lymphomas (1-4). It belongs to the flavin monoamine oxidase family, FIG1 subfamily. Enzymological characterization reveals that IL-4I1 has L-amino acid oxidase activity with preference toward aromatic amino acids. Studies have shown that hIL-4I1 inhibited the proliferation of CD3-stimulated T lymphocytes with a similar effect on CD4(+) and CD8(+) T cells (5). Its inhibitory effect was dependent on enzymatic activity and H_2O_2 production. Its restricted expression to lymphoid tissues indicates that it may play an important function in the immune system (1, 4).

References:

1. Chu, C.C. and W.E. Paul. (1997) Proc. Natl. Acad. Sci. USA **94**:2507.
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3. Chavan, S.S. *et al.* (2002) Biochim. Biophys. Acta. **1576**:70.
4. Copie-Bergman, C. *et al.* (2003) Blood **101**:2756.
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