Recombinant Human Adenosine Deaminase 2/CECR1

Catalog Number: 7518-AD

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Adenosine Deaminase 2/CECR1 protein Ile30-Lys511, with a C-terminal 6-His tag Accession # AAF65941
N-terminal Sequence Analysis	Ile30
Predicted Molecular Mass	57 kDa

SPECIFICATIONS

SDS-PAGE	58-66 kDa, reducing conditions
Activity	Measured by the ability to catalyze the hydrolytic deamination of adenosine to inosine. The specific activity is >14,000 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 HEPES, NaCl, Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Sodium phosphate, 1 M NaCl, pH 6.0
- Recombinant Human Adenosine Deaminase 2/CECR1 (rhCECR1) (Catalog # 7518-AD)
- Substrate: Adenosine (Sigma, Catalog # A9251), 10 mM stock in deionized water (incubate 10 minutes at 37 °C to fully solubilize)
- Stop/detection reagent: 0.2 M Sodium Hydroxide, 15 mM ortho-phthaldehyde (Sigma, Catalog # P0657), 0.1% β-mercaptoethanol
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhCECR1 to 0.5 µg/mL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. In plate, combine 50 µL dilute rhCECR1 with 50 µL dilute substrate. Include a substrate control containing 50 µL dilute enzyme only.
4. Incubate reactions at room temperature for 10 minutes.
5. Add 100 µL stop/detection reagent to all wells used. Add 50 µL substrate to substrate control wells.
6. Incubate for 30 minutes at room temperature in the dark to fully develop.
7. Read with excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.
8. Specific activity may be determined using the following equation:

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

*Adjusted for substrate control

**Derived using calibration standard ammonium sulfate (Amresco, Catalog # 0191).

Final Assay Conditions

Per Reaction:

- rhCECR1: 0.025 µg
- Adenosine: 1 mM

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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Adenosine deaminase is one of the key enzymes of purine nucleotide metabolism. It catalyses the conversion of adenosine and deoxy-adenosine to inosine and deoxy-inosine, respectively (1, 2). Adenosine Deaminase 2 (ADA2) is also known as CECR1 because it is a candidate gene for cat eye syndrome, a developmental disorder (3). ADA is a secreted protein that is expressed in many tissues, with the highest expression in lymphoblasts, heart, lung, and placenta (4). ADA2 is a member of a family of adenosine deaminase-related growth factors (ADGFs), proteins that are involved in tissue development (4). ADA2 induces the differentiation of monocytes into macrophages and stimulates the proliferation of T helper cells and macrophages by a mechanism independent of its catalytic activity (5). It has been suggested that ADA2 could be a therapeutic target for the control of immune responses in inflammation and cancer (5).

References:

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2. Lowenstein, J.M. (1972) Physiol. Rev. **52**:382.
3. Riazi, M.A. *et al.* (2000) Genomics **64**:277.
4. Zavialov, A.V. and A. Engstrom (2005) Biochem. J. **391**:51.
5. Zavialov, A.V. et al. (2010) J. Leucoc. Biol. **88**:279.

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