Recombinant Human Adenosine Deaminase/ADA

Catalog Number: 7048-AD

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Met1-Leu363, with a C-terminal 6-His tag Accession # P00813
N-terminal Sequence Analysis	Inconclusive result, Met predicted. Protein identity confirmed by MS analysis of tryptic fragments
Predicted Molecular Mass	42 kDa

SPECIFICATIONS

SDS-PAGE	40-42 kDa, reducing conditions
Activity	Measured by the ability to catalyze the hydrolytic deamination of adenosine to inosine. The specific activity is >44,000 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 Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, pH 7.5
- Recombinant Human Adenosine Deaminase/ADA (rhADA) (Catalog # 7048-AD)
- Substrate: Adenosine (Sigma, Catalog # A9251), 10 mM stock in deionized water
- UV plate (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Thaw the Substrate stock and then warm it at 37 °C for 20 minutes. Mix well after incubation.
2. Dilute rhADA to 0.1 ng/µL in Assay Buffer.
3. Dilute Substrate to 200 µM in Assay Buffer.
4. Load into a plate 50 µL of 0.1 ng/µL rhADA and start the reaction by adding 50 µL of 200 µM Substrate. For Substrate Blanks, load 50 µL of Assay Buffer and 50 µL of 200 µM Substrate.
5. Read plate at a wavelength of 265 nm (bottom read) in kinetic mode for 5 minutes.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x -1 x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 8500 $M^{-1}cm^{-1}$
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhADA: 0.005 µg
- Substrate: 100 µM

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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Adenosine Deaminase (ADA, adenosine aminohydrolase) is one of the key enzymes of purine nucleotide catabolism. It catalyses the hydrolytic deamination of adenosine and deoxy-adenosine to inosine and deoxyinosine (1, 2). ADA is expressed in virtually all tissues and is expressed at high levels in T-lymphocytes. Adenosine Deaminase deficiency can cause a form of SCID (severe combined immunodeficiency) and lymphopenia in both B- and T-cell lineages (3, 4). ADA can be used as a sensitive diagnostic marker for tuberculous pleuritis (5). Although it is primarily a cytosolic enzyme, ADA is known to be a positive regulator of T-cell co-activation due to its binding to CD26 at the cell surface. The interaction of ADA with CD26 regulates lymphocyte-epithelial cell adhesion (6).

References:

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