Recombinant Human PADI4

Catalog Number: 6660-AD

DESCRIPTION

Source	*E. coli*-derived Ala2-Pro663, with an N-terminal 11 kDa fusion protein, a thrombin cleavage linker and a C-terminal 6-His tag Accession # AAH25718
N-terminal Sequence Analysis	Inconclusive result, Met predicted. Protein identity confirmed by MS analysis of tryptic fragments
Predicted Molecular Mass	87 kDa

SPECIFICATIONS

SDS-PAGE	75-85 kDa, reducing conditions
Activity	Measured by its ability to catalyze the deimination of benzoyl-arginine ethyl ester. The specific activity is >1000 pmol/min/µg, as measured under the described conditions. www.RnDSystems.com.
Endotoxin Level	<1.0 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 Sodium Acetate, NaCl, DTT, EDTA and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.1 M NaCl, 10 mM $CaCl_2$, 5 mM DTT, pH 7.5
- Recombinant Human PADI4 (rhPADI4) (Catalog # 6660-AD)
- Benzoyl-arginine ethyl ester (BAEE) (Sigma, Catalog # B4500), 1 M stock in DMSO
- 2,3-Butanedione monoxime (DAMO) (Sigma, Catalog # B0753)
- Ammonium iron (III) sulfate dodecahydrate (Sigma, Catalog # 221260)
- Thiosemicarbazide (Sigma, Catalog # 89050)
- o-Phosphoric acid, 85% (Fisher, Catalog # A242)
- Sulfuric acid (Fisher, Catalog # A300)
- L-Citrulline (Sigma, Catalog # C7269), 20 mM stock in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare Color Developing Reagent A (80 mM DAMO, 2 mM Thiosemicarbazide) in deionized water.
2. Prepare Color Developing Reagent B (17.35% (v/v) Phosphoric Acid, 33.7% (v/v) Sulfuric Acid, 0.765 mg/mL Ammonium Iron Sulfate) in deionized water.
3. Dilute rhPADI4 to 4 ng/µL in Assay Buffer.
4. Dilute BAEE to 10 mM in Assay Buffer.
5. Dilute 20 mM L-Citrulline stock to 1000 µM in Assay Buffer. This is the first point of the standard curve.
6. Continue standard curve by performing six one-half serial dilutions of the 1000 µM L-Citrulline in Assay Buffer. The standard curve has a range of 0.625 to 40 nmol per well.
7. Pipet 150 µL of each point of the standard curve into microtubes.
8. Combine 75 µL of 4 ng/µL rhPADI4 with 75 µL of 10 mM BAEE in microtubes. Include a Substrate Blank containing Assay Buffer in place of rhPADI4.
9. Incubate reaction, blank, and standard curve for 30 minutes at 37 °C.
10. Prepare Color Developing Reagent C (1 part Reagent A : 3 parts Reagent B).
11. Add 600 µL of Color Developing Reagent C to reaction, blank, and standard curve tubes.
12. Heat the tubes at 95-100 °C in a heating block for 15 minutes.
13. Cool tubes at room temperature for 5 minutes.
14. Load 200 µL into wells.
15. Read plate at 540 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Product* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the L-Citrulline standard curve using linear fitting and adjusted for Substrate Blank.

Final Assay Conditions

Per Well:

- rhPADI4: 0.08 µg
- BAEE: 1 mM

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.

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BACKGROUND

Peptidyl-arginine deiminase-4 (PADI4) is a member of the peptide arginine deiminase family, which can catalyze the conversion of peptidyl arginine to citrulline in the presence of calcium ions (1). The process leads to the post-translational modification of proteins. Five isoforms of this family are present in vertebrates: PADI1, 2, 3, 4, and 6. PADI4 is predominantly expressed in blood lymphocytes and has been suggested to play a role in inflammation and the immune response. High expression of PADI4 has been detected in synovial tissues of rheumatoid arthritis (RA) patients producing autoantibodies that recognize citrulline containing proteins, and the enzyme is also present in monocytes, macrophages, eosinophils and neutrophils of the affected tissue (2). Thus, PADI4 is believed to play a causative role in RA. It also may be involved with transcription and apotosis by antagonizing the methylation of histones H2A, H3, and H4 via deimination which represses hormone (estrogen)-target transcription and interrupts cell apoptosis (3). It is a target gene of p53 in DNA damage response to induce protein citrullination (4). Its high expression in a variety of malignant tumors compared to normal tissues and benign tumors and its detection in the plasma of patients with malignant tumors makes it a potential tumor marker (5). It is also implicated in demyelinating diseases such as multiple sclerosis because of increased expression of PADI2 and PADI4 in myelin isolated from patients with MS (6).

References:

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