Recombinant Human Arginase 2/ARG2

Catalog Number: 9767-AR

DESCRIPTION

Source	*E. coli*-derived Val23-Ile354 Accession # P78540 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	37 kDa

SPECIFICATIONS

SDS-PAGE	40 kDa, reducing conditions
Activity	Measured by the production of urea during the hydrolysis of arginine. The specific activity is >60,000 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Diluent: Deionized Water
- Recombinant Human Arginase 2/ARG2 (rhARG2) (Catalog # 9767-AR)
- Urea, 100 mM stock in deionized water
- Substrate Buffer: 125 mM L-Arginine, 625 mM Glycine, pH 10.5
- Manganese Chloride, 1 M stock in deionized water
- O-Phthaldialdehyde (oPA), (Sigma, Catalog # P0657), 50 mg/mL (373 mM) stock in DMSO
- N-(1-Naphthyl)ethylene-diamine dihydrochloride (NED) (Sigma, Catalog # N9125), 500 mM stock in DMSO
- 50 mM Boric Acid, 1 M Sulfuric Acid, 0.03% Brij-35 (w/v) [Caution: highly acidic, neutralize before disposal]
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhARG2 to 0.8 µg/mL in deionized water.
2. Prepare a standard curve from the 100 mM Urea stock. Dilute 100 µL of 100 mM Urea with 900 µL of deionized water to make a 10 mM Urea solution. Use this for the first point of the curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 10 mM Urea stock in deionized water. The standard curve has a range of 7813 to 500,000 pmol per well.
4. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of deionized water.
5. Load 25 µL of to 0.8 µg/mL rhARG2 into empty wells of the same plate as the curve. Load multiple wells as some will be used as controls.
6. From the Substrate Buffer and Manganese Chloride stocks, prepare a solution of 60 mM Arginine, 300 mM Glycine, 40 µM MnCl2, pH 10.5. Do not prepare this solution until immediately before use as the manganese will gradually precipitate out of solution.
7. Add 25 µL of 60 mM Arginine, 300 mM Glycine, 40 µM MnCl2, pH 10.5 to the wells containing 0.8 µg/mL rhARG2 (exclude the controls). Mix well.
8. Cover the plate and incubate at 37° C for 30 minutes.
9. Dilute oPA stock to 4 mM in 50 mM Boric Acid, 1 M Sulfuric Acid, 0.03% Brij-35 (w/v).
10. Dilute NED stock to 4 mM in 50 mM Boric Acid, 1 M Sulfuric Acid, 0.03% Brij-35 (w/v).
11. Combine equal volumes of 4 mM oPA and 4 mM NED to form a solution of 2 mM oPA with 2 mM NED.
12. Add 200 µL of 2 mM oPA, 2 mM NED solution to all wells, including the standard curve.
13. Prepare a fresh solution of 60 mM Arginine, 300 mM Glycine, 40 µM MnCl2, pH 10.5. Add 25 µL to each well used as a control.
14. Cover the plate and incubate at room temperature for 20 minutes.
15. Read plate at 520 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Urea Detected* (pmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the urea standard curve using linear or 4-parameter fitting and adjusted for controls.

Final Assay Conditions

Per Reaction:

- rhARG2: 0.02 µg
- Arginine: 6 mM
- oPA & NED: 1.6 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

Arginase-2, a binuclear manganese-dependent metalloenzyme, is an approximately 36 kDa protein that forms a trimeric active molecule that catalyses the conversion of L-arginine into L-ornithine and urea (1). There are two isoforms of human arginase that share 58% sequence identity. Arginases are critical to the urea cycle and also influence downstream nitric oxide and polyamine levels (2) and hence are implicated to play a role in a number of diseases. While Arginase 1 is cytosolic and expressed predominantly in the liver, Arginase 2 is a mitochondrial enzyme expressed in several non-hepatic tissues including kidney, prostate and immune cells where it is regulated by inflammatory cytokines (2,3). Arginase 2 is implicated in regulation of a nitric oxide-dependent inflammatory response in lung and bronchial epithelial disease (4,5). It has been implicated in neuronal disease such as Huntington's disease due to nitric oxide signaling and suppressed enzymatic activity (6) and to play a role in atherosclerosis and hypertension through signaling pathways independent of its enzymatic activity (7,8). Arginase 2 promotes tumorigenesis through immune suppression, metabolism, cell proliferation and vascularization (9-11). Arginase-2 was noted as an attractive tumor therapeutic target (10) given that genetic deletion in mice does not lead to significant abnormalities (12).

References:

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