

Background

Arginase 1, also known as liver arginase, is a binuclear manganese metalloenzyme. It is a key enzyme of the urea cycle that catalyses the conversion of L-arginine into L-ornithine and urea, the final cytosolic reaction of urea formation in the mammalian liver (1). Arginase 1 is abundantly expressed in liver, but it is also expressed in cells and tissues that lack a complete urea cycle, including lung. Arginase is a critical regulator of nitric oxide synthesis and vascular function (2). It is implicated in a variety of human diseases including vascular disease, pulmonary disease, infectious disease, immune cell function and cancer (3). In humans, hereditary defects in arginase result in an accumulation of arginine in the blood known as hyperarginemia (4). Arginase deficiency can also result in the accumulation of nitrogen in the form of ammonia, which results in hyperammonemia (5).

References:

1. Dowling, D. *et al.* (2008) *Cell Mol. Life Sci.* **65**:2039.
2. Durante, W. *et al.* (2007) *Clin. Exp. Pharmacol. Physiol.* **34**:906.
3. Morris, S. (2009) *Br. J. Pharmacol.* **157**:922.
4. Crombez, E. *et al.* (2005) *Mol. Genet. Metab.* **84**:243.
5. Scaglia, F. *et al.* (2004) *J. Nutr.* **134**:2775s.

Description

Source	<i>E. coli</i> -derived Met1 - Lys322 with an N-terminal Met and 6-His tag Accession # P05089
N-terminal Sequence Analysis	Met
Structure / Form	Monomer
Predicted Molecular Mass	36 kDa

Specifications

SDS-PAGE	40 kDa, reducing conditions
Activity	Measured by the production of urea during the hydrolysis of arginine. The specific activity is > 35,000 pmoles/min/μg, as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 μg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 μm filtered solution in Tris, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

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. <ul style="list-style-type: none"> ● 6 months from date of receipt, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

Activity Assay Protocol

Materials

- Assay Diluent: Deionized Water
- Recombinant human ARG1 (R&D Systems, Catalog # 5868-AR)
- Substrate Buffer: 125 mM L-Arginine, 625 mM Glycine, pH 10.5
- Manganese Chloride, 1 M stock in deionized water
- o-Phthalaldehyde (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]
- Urea, 100 mM stock in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhARG1 to 0.25 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. Perform six additional one-half serial dilutions of the 10 mM Urea stock in deionized water. The standard curve has a range of 7813 to 500,000 pmoles per well.
4. Load 50 μL of each dilution of the standard curve into a plate. Include a blank containing only deionized water.
5. Load 40 μL of the 0.25 μg/mL rhARG1 into the plate. Load multiple wells as some will be used as enzyme blanks.
6. From the Substrate Buffer and Manganese Chloride stocks, prepare a solution of 100 mM Arginine, 500 mM Glycine, 1.25 mM MnCl₂, pH 10.5. Do not prepare this solution until immediately before use as the manganese will gradually precipitate out of solution.
7. Add 10 μL of 100 mM Arginine, 500 mM Glycine, 1.25 mM MnCl₂, pH 10.5 to the wells containing 0.25 μg/mL rhARG1 (exclude the blanks). Mix well.
8. Cover the plate and incubate at 37 °C for two hours.
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 the 2 mM oPA, 2 mM NED solution to all wells, including the standard curve.
13. Prepare a fresh solution of 100 mM Arginine, 500 mM Glycine, 1.25 mM MnCl₂, pH 10.5. Add 10 μL to each well used as an enzyme blank.
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:

$$\text{Specific Activity (pmoles/min/}\mu\text{g)} = \frac{\text{Adjusted Urea Detected* (OD)}}{\text{Incubation time (min) x amount of enzyme (}\mu\text{g)}}$$

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

Final Assay Conditions Per Well

- rhARG1: 0.01 μg (10 ng)
- Arginine: 4 mM
- oPA & NED: 1.6 mM
- Urea Curve: 500000, 250000, 125000, 62500, 31250, 15625 and 7813 pmoles

12/3/2009

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