QuantiChrom™ Arginase Assay Kit (DARG-100)

Quantitative Colorimetric Arginase Determination

DESCRIPTION

ARGINASE (L-arginine ureohydrolase EC 3.5.3.1) is present in mammals and plants. In humans, arginase is expressed predominantly in the liver, and to lesser degrees in breast, kidney, testes, salivary glands, epidermis and erythrocytes. Arginase catalyzes the conversion of arginine to ornithine and urea, completing the last step in the urea cycle. Arginase activity is a key diagnostic indicator. Increased levels of arginase activity in blood have been associated with liver damage. Hyperargininemia due to arginase deficiency is an inherited autosomal recessive disease.

Simple, direct and automation-ready procedures for measuring arginase activity in biological samples are highly desirable in Research and Drug Discovery. BioAssay Systems' arginase assay kit provides a sensitive and convenient method for arginase activity determination. The method utilizes a chromogen that forms a colored complex specifically with urea produced in the arginase reaction. The intensity of the color is directly proportional to the arginase activity in the sample.

KEY FEATURES

Sensitive and accurate. Detection limit: 0.3 U/L for 2 hr arginase reaction in 96-well assay format.

Simple and high-throughput. The procedure involves incubation of the provided substrate with the sample in a microplate followed by the addition of the coloring reagent. Can be readily automated as a high-throughput assay for thousands of samples per day.

APPLICATIONS

Direct Assays: arginase activity in enzyme preparations, serum, plasma, tissue culture etc;

Drug Discovery/Pharmacology: effects of drugs on arginase activity.

KIT CONTENTS

Arginine Buffer (pH 9.5):	1.5 mL	**Mn Solution:**	300 µL
Reagent A:	12 mL	**Reagent B:**	12 mL
Urea standard (50 mg/dL):	0.5 mL		

Storage conditions. Kit is shipped at room temperature. Store the Arginine Buffer and Urea Standard at -20°C, and other components at 2-8°C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

SAMPLE PREPARATION

Serum and Plasma samples contain urea. Urea can be depleted using a membrane filter (e.g. Amicon Ultra-0.5, Ultracel-10 Membrane, 10 kDa from Millipore) with the following procedure:

1. Load up to 100 µL sample in an Amicon Ultra-0.5 (10 kDa cutoff) and dilute with water to 500 µL. Centrifuge at 14000 rpm for 30 min, check level of sample, ideally the sample level will be less than 50 µL. Add water to 500 µL and repeat the centrifugation.
2. Decant concentrated sample diluent and measure final volume with a pipetman. Adjust final volume so there will be enough sample for the reaction and reaction blank.

Cell Lysates: Harvest ~10^6 cells per sample and wash with PBS. Centrifuge at 1,000*g* at 4°C for 10 min. Lyse cell pellets for 10 min in 100 µL of 10 mM Tris-HCl (pH 7.4) containing 1 µM pepstatin A, 1 µM leupeptin, and 0.4% (w/v) Triton X-100. Centrifuge lysates at 14,000*g* at 4°C for 10 min. Use supernatant for arginase assay.

ASSAY PROCEDURE

Bring all reagents to room temperature prior to assay. Arginine Buffer should be preheated to 37°C. *Important*: use reconstituted reagents within 2 hours after preparation.

1. *Urea Standard*. Prepare 1 mM Urea Standard by mixing 24 µL 50 mg/dL urea and 176 µL water. Add 50 µL 1 mM Urea Standard and 50 µL dH_2O to separate wells of a 96 well plate.
2. *Arginase Reaction*: Prepare *5× Substrate Buffer* by combining 4 vol of Arginine Buffer and 1 vol of the Mn Solution. For each test, 10 µL *5× Substrate Buffer* is needed. Next, add 40 µL of each sample to 2 separate wells of a 96 well plate. Add 10 µL 5× Substrate Buffer into one of the sample wells (OD_{SAMPLE}). Leave the other sample well **without** 5× Substrate Buffer (Sample Blank Control, OD_{BLANK}). Incubate reaction plate at 37°C for 2 hours or desired reaction time.

   Note: samples may need to be diluted with water depending on arginase activity. Although the assay is linear from 0.3-20 U/L for 2 hr arginase reaction, the assay works best if samples are diluted so apparent activities lie between 1 and 10 U/L.
3. *Urea Determination*: Prepare *Urea Reagent* by combining equal volumes of Reagent A and Reagent B. Add 200 µL *Urea Reagent* to all wells. (*note: Urea Reagent stops arginase reaction*) and then add 10 µL *5× Substrate Buffer* to the *Sample Blank Control* well. Tap the plate to mix. Incubate 60 min at room temperature and read optical density at 430nm.

Note: for some samples addition of urea reagent may cause turbidity. If this occurs, transfer sample to an Eppendorf tube and centrifuge for 5 minutes at 14,000 rpm. Transfer supernatant back to reaction plate and read the absorbance.

CALCULATION

Arginase activity (units per liter of sample (U/L)) is calculated as follows:

$$\text{Arginase} = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{WATER}} \times [\text{Urea Standard}] \times 50 \times 10^3 / (40 \times t)$$

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{WATER}} \times 10.4\ (\text{U/L})$$

where OD_{SAMPLE}, OD_{BLANK}, $OD_{STANDARD}$ and OD_{WATER} are the optical density values of the sample, sample blank, standard and water respectively. [Urea Standard] = 1 mM, *t* is the reaction time (120 min). 50 and 40 are the reaction and sample volumes (µL) respectively.

The incubation time for the arginase reaction can vary (0.5 to 4 hours) depending on the arginase activity. If $(OD_{SAMPLE} - OD_{BLANK})/(OD_{STANDARD} - OD_{WATER})$ is larger than 2, either dilute sample in distilled water and repeat the assay multiplying the results by the dilution factor or use a shorter arginase reaction time.

Unit definition: 1 unit of arginase converts 1 µmole of L-arginine to ornithine and urea per minute at pH 9.5 and 37°C.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories, clear bottom 96-well plates, plate reader and, for plasma and serum, Amicon Ultra-0.5, Ultracel-10 Membrane.

PUBLICATIONS

1. Moertel, L. et al. (2008) Comparative real-time PCR and enzyme analysis of selected gender-associated molecules in Schistosoma japonicum. Parasitology 135, 575–583.
2. Pulichino, A.M. et al. (2008) Identification of Transforming Growth Factor β1–Driven Genetic Programs of Acute Lung Fibrosis. Am. J. Respiratory Cell & Mol. Biol. 39: 324-336.
3. Ndolo, E.A. et al. (2010) The Role of Lysosomes in Limiting Drug Toxicity in Mice. J Pharmacol Exp Ther. 333:120-128.