

QuantiChrom™ Arginase Assay Kit (DARG-200)

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 [1]. Hyperargininemia due to arginase deficiency is an inherited autosomal recessive disease [2].

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: 1 U/L arginase activity in 96-well assay format.

Simple and high-throughput. The procedure involves incubation of the provided substrate with the sample in a microplate, addition of the coloring reagent and incubation for 15 min. 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 (for 200 samples in 96-well assay)

Arginine Buffer (pH 9.5): 2 mL **Mn Solution: 1 mL**
Reagent A: 25 mL **Reagent B: 25 mL**
Urea standard: 1 mL 50mg/dL

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: at least 6 months (see expiry dates on labels).

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.

PROCEDURES

Reagent Preparation: bring reagents to room temperature prior to assay.

5x Substrate Buffer: combine 4 vol of Arginine Buffer and 1 vol of the Mn Solution. For each test, 10 µL 5x Substrate Buffer is needed.

Urea Reagent: combine equal volumes of Reagent A and Reagent B.

1 mM Urea Standard: mix 24 µL 50mg urea /dL and 176 µL water.

Important: use the above reagents within 2 hours after preparation.

Standard procedure using 96-well plate (200 tests):

1. **Arginase Reaction:** combine 40 µL sample and 10 µL 5x Substrate Buffer into wells of a clear bottom 96-well reaction plate. In addition, transfer 40 µL sample **without** 5x Substrate Buffer (Sample Blank Control, OD_{BLANK}), 50 µL H₂O (standard background, OD_{WATER}) and 50 µL 1 mM Urea Standard (OD_{STANDARD}) into separate wells of the reaction plate and incubate at 37°C for 2 hours.

Note: samples may need to be diluted with water depending on arginase activity. Assay works best if sample is diluted so apparent activity lies between 1 and 40 U/L. Serum or plasma samples contain urea which may need to be removed prior to assaying (see General Considerations).

2. **Urea Determination:** Add 200 µL Urea Reagent to all wells (**note: Urea Reagent stops arginase reaction**) and then add 10 µL 5x Substrate Buffer to the **Sample Blank Control** well. Tap the plate to mix.

3. Incubate 60 min at room temperature and read optical density at 430nm or incubate 20 min for measurement at 520nm.

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 14000 rpm. Transfer supernatant back to reaction plate and read the absorbance.

CALCULATION

Arginase activity (units per liter of sample) is calculated as

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

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

OD_{SAMPLE}, OD_{BLANK}, OD_{STANDARD} and OD_{WATER} are optical density values of 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.

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.

GENERAL CONSIDERATIONS

A. The incubation time for the arginase reaction (Step 1) can vary (0.5 to 4 hours) depending on the arginase activity. If (OD_{SAMPLE} - OD_{BLANK})/(OD_{STANDARD} - OD_{WATER}) is larger than 3.5, dilute sample in distilled water and repeat the assay, multiply the results by the dilution factor.

B. **Sample Pretreatment:** serum or plasma samples contain urea. Urea can be depleted using a membrane filter (e.g. Microcon YM-10 from Millipore). The recommended procedure,

1. Load up to 100 µL sample in a Microcon YM-10 (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.

C. **Cell Lysate.** ~10⁶ cells per sample are harvested, washed with PBS, and centrifuged at 1000g at 4°C for 10 min. Pellets are lysed for 10 min in 100 µL of 10 mM Tris-HCl (pH 7.4) containing 0.15 mM pepstatin A, 0.2 mM leupeptin, and 0.4% (w/v) Triton X-100. Samples are centrifuged at 20,000g at 4°C for 10 min. Use supernatant for arginase assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories, clear bottom 96-well plates, plate reader and for plasma and serum, Millipore Microcon YM-10.

EXAMPLES

A rat serum sample was assayed in duplicate using the standard 96-well protocol. The arginase activity was 322 ± 5 U/L. An undiluted human serum from a healthy donor had an arginase activity of 0.88 ± 0.02 U/L.

LITERATURE

- [1]. Ugarte G, Pino M E, Peirano P, Marusic E. (1960) Serum arginase activity in subjects with hepatocellular damage. J Lab Clin Med. 55:522-9.
- [2]. Crombez EA, Cederbaum SD (2005) Hyperargininemia due to liver arginase deficiency. Mol Genet Metab. 84(3): 243-51.
- [3]. Mellerup B (1967) Colorimetric method for rapid determination of serum arginase. Clin Chem. 13(10): 900-8.