Technical Bulletin

Arginase Assay Kit

Catalogue number MAK533

Product 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. The 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.

The detection limit of the kit is 0.3 U/L for a 2-hour arginase reaction in 96-well assay format. The kit is used to determine the arginase activity in enzyme preparations, serum, plasma, and tissue culture.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Arginine Buffer (pH 9.5) 1.5 mL
  Catalogue Number MAK533A
- Mn Solution 300 mL
  Catalogue Number MAK533B
- Reagent A 12 mL
  Catalogue Number MAK533C
- Reagent B 12 mL
  Catalogue Number MAK533D
- Urea Standard (50 mg/dL) 0.5 mL
  Catalogue Number MAK533E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Amicon Ultra-0.5, Ultracel-10 Membrane
- 10 mM Tris-HCl (pH 7.4) containing 1 µM pepstatin A, 1 µM leupeptin, and 0.4% (w/v) Triton™ X-100

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at -20 °C.

Preparation Instructions

Equilibrate all components to room temperature prior to use. Briefly centrifuge small vials prior to opening.

Arginine Buffer**:** Buffer should be preheated to 37 °C.

Note: Use reconstituted reagents within 2 hours after preparation.

Sample Preparation

Serum and Plasma samples: Serum and Plasma Samples contain urea. Urea can be depleted using a membrane filter (for example: 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 14,000 rpm for 30 minutes, 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:

1. Harvest ~ 10^6 cells per Sample and wash with PBS.
2. Centrifuge at 1000 X g at 4 °C for 10 minutes.
3. Lyse cell pellets for 10 minutes 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.
4. Centrifuge lysates at 14,000 X g at 4 °C for 10 minutes.
5. Use supernatant for arginase assay.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of 1 mM Urea Standard

1. Mix 24 µL 50 mg/dL Urea Standard with 176 µL of purified water.
2. Add 50 µL 1 mM Urea Standard and 50 µL purified H_2O to separate wells of a 96 well plate.

Arginase Reaction

Prepare 5x Substrate Buffer by combining 4 volumes of Arginine Buffer and 1 volume of Mn Solution. Scale as needed. For each test, 10 µL 5x Substrate Buffer is needed.

1. Add 40 µL of each Sample to 2 separate wells of a 96 well plate.
2. Add 10 µL 5x Substrate Buffer into one of the Sample wells (OD_{SAMPLE}).
3. Leave the other Sample well without 5x Substrate Buffer (Sample Blank Control, OD_{BLANK}).
4. 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-hour arginase reaction, the assay works best if Samples are diluted so apparent activities lie between 1 and 10 U/L.

Urea Determination

Preparation of Urea Reagent: Prepare Urea Reagent by combining equal volumes of Reagent A and Reagent B. Prepare enough reagent for the assay.

Note: Urea Reagent stops the arginase reaction.

1. Add 200 µL Urea Reagent to all wells.
2. Add 10 µL 5x Substrate Buffer to the Sample Blank Control well.
3. Tap the plate to mix.
4. Incubate for 60 minutes at room temperature.
5. Read optical density (OD) of each well 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.

Results

Arginase activity (units per liter of sample (U/L)) is calculated as below.

$$\text{Arginase (U/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Water}} \times [\text{Urea Standard}] \times 50 \times 10^3 / (40 \times t)$$

$$\frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Water}} \times 10.4$$

Where:
OD_{SAMPLE} = OD values of the Sample

OD_{BLANK} = OD values of the Blank

$OD_{STANDARD}$ = OD values of the Standard

OD_{WATER} = OD values of the Water

[Urea Standard] = 1 mM

t = Reaction Time (120 min)

50 = reaction volumes (µL)
40 = sample volumes (µL)

Note: 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.

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