Technical Bulletin

Arginase Activity Colorimetric Assay Kit

Catalog Number MAK384

Product Description

Arginase is the final enzyme of the Urea Cycle. It converts L-arginine into urea and L-ornithine and participates in removing ammonium ion from the body. Arginase has two isoforms: Arginase I and Arginase II. Arginase I is mainly present in the liver and plays an important part in the urea cycle, whereas Arginase II is present in kidney and other tissues and regulates Arginine/Ornithine concentration. Arginase deficiency can lead to severe symptoms including neurological impairment, dementia, and hyperammonemia. Analysis of Arginase activity is fundamental to the study of the urea metabolic pathway.

The Arginase Activity Colorimetric Assay kit is simple, sensitive and rapid. In this method, Arginase reacts with arginine and undergoes a series of reactions to form an intermediate that reacts stoichiometrically with the Probe provided in the kit. The generated end-product is measured colorimetrically at 570 nm. The kit can detect Arginase activity less than 1 mU/well.

The kit is suitable for the determination of arginase activity in tissues (liver, heart, kidney, etc.) and cell culture samples (adherent and suspension cells).

Components

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

- Arginase Assay Buffer — 25 mL
  Catalog Number MAK384A
- Probe (in DMSO) — 200 µL
  Catalog Number MAK384B
- Arginase Substrate — 1 vial
  Catalog Number MAK384C
- Arginase Enzyme Mix — 1 vial
  Catalog Number MAK384D
- Arginase Developer — 1 vial
  Catalog Number MAK384E
- Arginase Converter Enzyme — 1 vial
  Catalog Number MAK384F
- H_2O_2 Standard (0.88 M) — 100 µL
  Catalog Number MAK384G
- Arginase Positive Control — 1 vial
  Catalog Number MAK384H

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96 well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

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 on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Arginase Assay Buffer: Warm Arginase Assay Buffer to room temperature prior to use. Chill an appropriate amount of Arginase Assay Buffer for use in Sample Preparation.

Probe (in DMSO): Ready to use as supplied. Aliquot and store at -20 °C. Warm to room temperature prior to use. Use aliquot within two months.

Arginase Substrate: Reconstitute with 220 µL of purified water. Store at -20 °C. Keep on ice while in use. Use within two months of reconstitution.

Arginase Enzyme Mix, Arginase Developer and Arginase Converter Enzyme: Reconstitute with 220 µL of Arginase Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20 °C. Keep on ice while in use. Use within two months of reconstitution.

Arginase Positive Control: Reconstitute with 100 µL of Arginase Assay Buffer and dissolve completely. Aliquot and store at -20 °C. Keep on ice while in use. Use within two months of reconstitution.

Procedure

Sample Preparation

Note: High urea content in samples will interfere with the assay. To remove urea from samples, use a 10 kDa spin column. Add 50-200 µL of sample into a spin column. Centrifuge at 15,000 × *g* for 2 minutes. Replenish the lost liquid and repeat 2 times, discarding the filtrate, and bringing the sample (retentate) to its original volume with Arginase Assay Buffer.

1. Homogenize tissue (10 mg) or cells (1×10^6 cells) with 100 µL of ice-cold Arginase Assay Buffer on ice.
2. Centrifuge at 10,000 × *g* for 5 minutes.
3. Collect the supernatant.
4. Add 1-40 µL of Sample(s) per well. For unknown samples, test different amounts of sample to ensure the readings are within the Standard Curve range.
5. Adjust the total volume to 40 µL with Arginase Assay Buffer.
6. For samples with high background, prepare parallel sample well(s) as the Sample Background Control to correct for interference from the urea in the sample.

Positive Control

Add 0.2-2 µL of Arginase Positive Control into the desired well(s) and adjust the total volume to 40 µL with Arginase Assay Buffer.

Substrate Mix Preparation

1. Mix enough reagents for the number of assays to be performed. For each Sample and Positive Control well, prepare 10 µL of Substrate Mix according to Table 1. Mix well.

Table 1.
Preparation of Substrate Mix

Reagent	Volume
Arginase Assay Buffer	8 µL
Arginase Substrate	2 µL

2. Add 10 µL of Substrate Mix to Sample(s) and Positive Control wells. Mix well.
3. For samples having high background (See Sample Preparation Step 6), add 10 µL of Arginase Assay Buffer to Sample Background Control well(s). Mix well.
4. Incubate for 20 minutes at 37 °C.

Standard Curve Preparation

Note: Diluted H_2O_2 is **unstable**, prepare fresh dilution for each set of assays.

1. Prepare a 10 mM solution of H_2O_2 by diluting 4 µL of H_2O_2 Standard (0.88 M) with 348 µL of purified water, mix well.
2. Prepare a 1 mM H_2O_2 solution by diluting 100 µL of the 10 mM H_2O_2 solution from Step 1 with 900 µL of purified water, mix well.
3. Prepare H_2O_2 Standards according to Table 2. Mix well.

Table 2.
Preparation of H_2O_2 Standard Standards

Well	1 mM H_2O_2 Standard	Purified Water	H_2O_2 (nmol/ well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Reaction Mix Preparation

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Reaction Mix according to Table 3.

Table 3.
Preparation of Reaction Mix

Reagent	Volume
Arginase Assay Buffer	42 µL
Arginase Enzyme Mix	2 µL
Arginase Developer	2 µL
Arginase Converter Enzyme	2 µL
Probe (in DMSO)	2 µL

2. Add 50 µL of Reaction Mix to each well containing Standards, Positive Control, test Samples, and Sample Background Control(s). Mix well.

Measurement

Measure absorbance at 570 nm (A_{570}) immediately in kinetic mode for 10-30 minutes at 37 °C.

Note: Incubation time depends on the Arginase Activity in the samples. Measure A_{570} in kinetic mode and choose two time points (T_1 and T_2) in the linear range to calculate the Arginase Activity of the samples. The H_2O_2 Standard Curve can be read in endpoint mode at the end of the incubation time.

Results

1. Subtract 0 Standard reading from all Standard readings.
2. Plot the H_2O_2 Standard Curve.
3. Correct Sample reading by subtracting the value derived from the Sample Background Control reading from Sample reading.
4. Calculate the Arginase Activity of the test samples: $\Delta A_{570} = A_2 - A_1$.
5. Apply the ΔA_{570} to the H_2O_2 Standard Curve to get B nmol of H_2O_2 generated by Arginase during the reaction time ($\Delta T = T_2 - T_1$).

Arginase Activity (nmol/min/µl = mU/µL or U/mL) =

$$[B/(\Delta T \times V)] \times \text{Dilution Factor}$$

where:

B = Amount of H_2O_2 amount from Standard Curve (nmol)

ΔT = Reaction time (minutes)

V = Sample volume added into the reaction well (µL)

Unit Definition: One unit of Arginase is the amount of enzyme that will generate 1.0 µmol of H_2O_2 per minute at pH 8 at 37 °C.

Figure 1.
Typical H_2O_2 Standard Curve

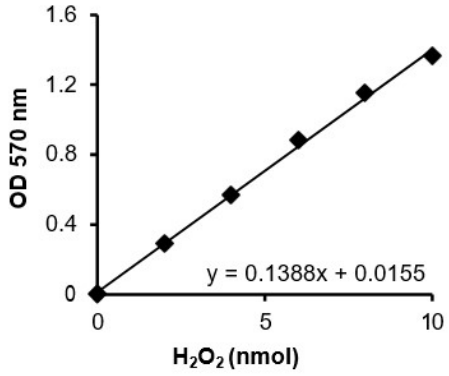
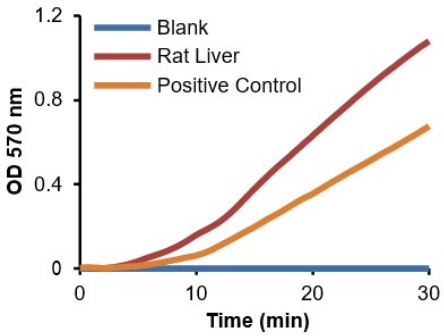


Figure 2.
Arginase activity in rat liver lysate (3 µg) and Positive Control (2 µL). Assays were performed following the kit protocol.



Frequently Asked Questions

Can catalase in the tissue lysates interfere with the assay by consuming the H_2O_2?

Endogenous enzymes in the samples, like catalase, can interfere with the assay. However, this will depend on the relative abundance of catalase versus arginase as well as their individual K_m values. According to experimental data, arginase activity in liver samples was detected higher than that of the background.

Can an alternate buffer be used for sample preparation (cell lysis, sample dilutions, etc.)?

The Arginase Activity Colorimetric Assay kit assay buffer is optimized for the reactions the kit is designed for. The buffer not only contains detergents for efficient lysis of cells/tissue, but also contain proprietary components required for the further reactions. Therefore, it is highly recommended to use the buffers provided in the kit for the best results.

Can serum samples be tested?

Under normal conditions, serum contains low levels of Arginase. However, serum can be tested directly by adding 1-40 µL per well.

Can frozen samples be used with this assay?

Fresh samples are recommended. However, frozen samples can be used provided they have not undergone multiple freeze-thaw cycles.

Does the kit work with bacteria or yeast cells?

The kit has been standardized for mammalian cells only.

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