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Product Information

Arginase Activity Assay Kit

Catalog Number **MAK112**

TECHNICAL BULLETIN

Product Description
Arginase is a manganese-containing enzyme that catalyzes the conversion of arginine to urea and ornithine. Two isoforms of arginase are present in most mammals, which differ in their tissue distribution and subcellular localization. Arginase I is a cytoplasmic protein predominantly expressed in the liver where it catalyzes the fifth and final step of the Urea Cycle. Arginase II is a mitochondrial protein with broader tissue distribution whose function may include nitric oxide and polyamine metabolism. Decreased activity or expression of Arginase I results in the autosomal recessive disorder hyperarginemia. Increased serum arginase activity is implicated in hepatic injury and in certain pathological conditions such as cancer.[1,2] Arginase also participates in multiple inflammatory reactions in the immune system.

The Arginase Activity Assay kit provides a simple and direct procedure for measuring arginase activity in a variety of samples such as enzyme preparations, serum, plasma, and tissue culture. In this assay, arginase catalyzes the conversion of arginine to urea and ornithine. The urea produced specifically reacts with the color development reagent to generate a colored product proportional to the arginase activity present. One unit of arginase is the amount of enzyme that will convert 1.0 μmole of L-arginine to ornithine and urea per minute at pH 9.5 and 37 °C. This kit has a detection limit of 0.3 unit/L for the 2 hour arginase reaction in a 96 well format.

Components
The kit is sufficient for 200 assays in 96 well plates.

Arginine Buffer, pH 9.5 2 mL
Catalog Number MAK112A

Reagent A 25 mL
Catalog Number MAK112B

Urea Standard, 50 mg/dL 1 mL
Catalog Number MAK112C

Mn Solution 1 mL
Catalog Number MAK112D

Reagent B 25 mL
Catalog Number MAK112E

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off Filter (Catalog Number Z677108 or equivalent)
- Reagents for cell lysing: Proteinase inhibitors Pepstatin A (Catalog Number P5318 or equivalent) and Leupeptin (Catalog Number L9783 or equivalent), Triton™ X-100 (Catalog Number T8787 or equivalent)

Preparation Instructions
Briefly centrifuge vials before opening. Bring all reagents to room temperature prior to assay. Use ultrapure water for the preparation of samples and reagents.
Note: Use all reconstituted reagents within 2 hours of preparation.

Arginine Buffer – Preheat buffer to 37 °C just before use.

Urea Standard Working Solution– Dilute 24 μL of the 50 mg/dL Urea Standard with 176 μL of water to prepare a 1 mM Standard Working Solution. Aliquot remaining 50 mg/dL Urea Standard solution and store at –20 °C.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
This kit is shipped at room temperature. Store the Arginine Buffer and Urea Standard at –20 °C. All other components are stored at 2–8 °C.

To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Procedure
Sample Preparation
Serum and plasma sample preparation – These samples contain urea. Urea can be depleted using a 10 kDa Molecular Weight Cut-Off Filter.

Load up to 100 µL of a serum/plasma sample in cut-off filter and dilute with water to 500 µL. Centrifuge the samples at 14,000 × *g* for 30 minutes. Check level of sample, ideally the sample level will be less than 50 µL. Add water to 500 µL and repeat centrifugation.

Decant concentrated sample and measure final volume with a pipette. Adjust final volume so there will be enough sample for the reaction and sample blank.

Cell lysate preparation – Harvest ~1 × 10^6 cells and wash with PBS. Centrifuge at 1,000 × *g* for 10 minutes at 4 °C. Lyse cells 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. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Add up to 40 µL of sample supernatant to each of two wells of a 96 well plate, one each for the sample well and the sample blank well. Bring each well to a final volume of 40 µL with water.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the assay. Although the assay is linear from 0.3–20 units/L for the 2 hour arginase reaction, the assay works best if samples are diluted so apparent activity lies between 1–10 units/L.

Urea Standard
Add 50 µL of prepared 1 mM Standard Working Solution and 50 µL of water to separate wells of a 96 well plate.

Assay Reaction
1. Prepare the 5× Substrate Buffer according to the scheme in Table 1. 10 µL of the 5× Substrate Buffer is required for each sample and sample blank well.

Table 1.
5× Substrate Buffer

Reagent	Volume
Arginine Buffer	8 µL
Mn Solution	2 µL

2. Add 10 µL of the 5× Substrate Buffer to each of the sample wells. Do not add to the sample blank wells. Mix well using a horizontal shaker or by pipetting. Incubate the plate for 2 hours at 37 °C. Cover the plate during the incubation.

3. Prepare Urea Reagent according to the scheme in Table 2. 200 µL of the Urea Reagent is required for each well (Urea Standard, water, sample, and sample blank wells).

Table 2.
Urea Reagent

Reagent	Volume
Reagent A	100 µL
Reagent B	100 µL

Note: Reagent B may be yellow in color

4. To stop the arginase reaction, add 200 µL of the prepared Urea Reagent to each well (Urea Standard, water, sample, and sample blank wells). Then add 10 µL of the 5× Substrate Buffer to the sample blank wells. Tap the plate to mix. Incubate plate for 60 minutes at room temperature.
   Note: For some samples, the addition of the Urea Reagent may cause turbidity. If this occurs, transfer sample to a microtube and centrifuge for 5 minutes at 13,000 × *g*. Transfer supernatant back to plate and read the absorbance.

5. Measure the absorbance at 430 nm (A_{430}).

Calculations

The Arginase activity of a sample may be determined by the following equation:

$$\text{Activity} = \frac{(A_{430})_{sample} - (A_{430})_{blank}}{(A_{430})_{standard} - (A_{430})_{water}} \times \frac{(1\ mM \times 50 \times 10^3)}{(V \times T)}$$

T = Reaction time in minutes
V = sample volume (µL) added to well (1–40 µL)
1 mM = concentration of Urea Standard
50 = reaction volume (µL)
10^3= mM to µM conversion factor

Note: The incubation time for the arginase reaction can vary between 0.5–4 hours, depending on the arginase activity. If the ratio between the corrected sample $[(A_{430})_{sample} - (A_{430})_{blank}]$ and corrected standard $[(A_{430})_{standard} - (A_{430})_{water}]$ is larger than 2, either dilute sample in water and repeat the assay, or use a shorter reaction time.

One unit of Arginase is the amount of enzyme that will convert 1.0 µmole of L-arginine to ornithine and urea per minute at pH 9.5 and 37 °C.

Sample Calculation:

$(A_{430})_{sample}$ = 1.8
$(A_{430})_{blank}$ = 0.7
$(A_{430})_{standard}$ = 1.3
$(A_{430})_{water}$ = 0.5

T = 120 minutes
V = 40

Arginase activity is:

$$\frac{1.8 - 0.7}{1.3 - 0.5} \times \frac{(1\ mM \times 50\ \mu L \times 10^3)}{(40\ \mu L \times 120\ min)}$$

1.375 × 10.4 units/L = 14.3 units/L

References

1. Jeyabalan, Geetha, *et al*., Arginase blockade protects against hepatic damage in warm ischemia-reperfusion. *Nitric oxide: biology and chemistry,* vol. 19,1 (2008).
2. Polat, M., *et al.,* Elevated Serum Arginase Activity Levels in Patients with Breast Cancer. *Surg. Today,* **33**, 655–661 (2003).

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Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at 37 °C
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in incompatible buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare reagents fresh for each assay
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further