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. 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
  Catalog Number MAK112A
  2 mL

- Reagent A
  Catalog Number MAK112B
  25 mL

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

- Mn Solution
  Catalog Number MAK112D
  1 mL

- Reagent B
  Catalog Number MAK112E
  25 mL

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
This product is 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine Buffer</td>
<td>8 μL</td>
</tr>
<tr>
<td>Mn Solution</td>
<td>2 μL</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>100 μL</td>
</tr>
<tr>
<td>Reagent B</td>
<td>100 μL</td>
</tr>
</tbody>
</table>

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:

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

T = Reaction time in minutes
V = sample volume (\(\mu\)L) added to well (1–40 \(\mu\)L)
1 mM = concentration of Urea Standard
50 = reaction volume (\(\mu\)L)
10^3 = mM to \(\mu\)M 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})_{\text{sample}} - (A_{430})_{\text{blank}}]/[(A_{430})_{\text{standard}} - (A_{430})_{\text{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 \(\mu\)mole of L-arginine to ornithine and urea per minute at pH 9.5 and 37 °C.

Sample Calculation:

\[
(A_{430})_{\text{sample}} = 0.75 \\
(A_{430})_{\text{blank}} = 0.07 \\
(A_{430})_{\text{standard}} = 0.54 \\
(A_{430})_{\text{water}} = 0.04 \\
T = 120 \text{ minutes} \\
V = 40
\]

Arginase activity is:

\[
0.75 - 0.07 \times (1 \text{ mM} \times 50 \text{ \(\mu\)L} \times 10^3) \\
0.54 - 0.04 \frac{(40 \text{ \(\mu\)L} \times 120 \text{ min})}{1.36 \times 10.4 \text{ units/L} = 14.1 \text{ units/L}}
\]
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Cold assay buffer</td>
<td>Assay Buffer must be at 37 °C</td>
</tr>
<tr>
<td></td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
</tr>
<tr>
<td></td>
<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Type of 96 well plate used</td>
<td>For colorimetric assays, use clear plates</td>
</tr>
<tr>
<td>Samples with erratic readings</td>
<td>Samples prepared in incompatible buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
</tr>
<tr>
<td></td>
<td>Cell/Tissue culture samples were incompletely homogenized</td>
<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
</tr>
<tr>
<td>Lower/higher readings in samples and standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
</tr>
<tr>
<td></td>
<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare reagents fresh for each assay</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
</tr>
<tr>
<td></td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
</tr>
</tbody>
</table>

Triton is a trademark of The Dow Chemical Company or an affiliated company of Dow.

KVG,LS,MAM 02/18-1