Instructions for Use

For the rapid, sensitive and accurate measurement of Arginase in various samples.

This product is for research use only and is not intended for diagnostic use.
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INTRODUCTION

1. BACKGROUND

Arginase Activity Assay Kit (colorimetric) (ab180877) is a simple, sensitive and rapid product to quantify arginase activity. In this assay, arginase reacts with arginine and undergoes a series of reactions to form an intermediate that reacts stoichiometrically with the probe to generate a colored product that can be detected at OD = 570 nm. The kit can detect less than 0.2 U/L of arginase activity in 96-well assay format.

Arginase is the final enzyme of the urea cycle. It converts L-arginine into urea and L-ornithine and plays an important role 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.
2. **ASSAY SUMMARY**

- Standard curve preparation
- Sample preparation*
  - Add substrate mix and incubate 37°C for 20 min
  - Add reaction mix
  - Measure optical density (OD570 nm) in a kinetic mode
    - 37°C for 10 - 30 min**

*Samples might require deproteinization.

**For kinetic mode detection, incubation time given in this summary is for guidance only.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**
5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginase Assay Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>OxiRed™ probe (in DMSO)</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Arginase Substrate (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Arginase Enzyme Mix (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Arginase Developer (lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Arginase Converter Enzyme</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>(lyophilized)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O2 Standard (0.88 M)</td>
<td>100 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Arginase Positive Control</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>(lyophilized)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD570 nm
- 96 well plate: clear plates for colorimetric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)
- 10 kD Spin Columns (ab93349)
7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
8. **TECHNICAL HINTS**

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Keep enzymes, heat labile components and samples on ice during the assay.

- Make sure all buffers and solutions are at room temperature before starting the experiment.

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Ensure plates are properly sealed or covered during incubation steps.

- Make sure you have the right type of plate for your detection method of choice.

- Make sure the heat block/water bath and microplate reader are switched on.
9. **REAGENT PREPARATION**

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Arginase Assay Buffer:**

   Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C. Use within 2 months.

9.2 **OxiRed Probe – in DMSO:**

   Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE:** DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C. Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Once the probe is thawed, use with two months.

9.3 **Arginase Substrate:**

   Reconstitute in 220 µL ddH₂O. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.4 **Arginase Enzyme Mix:**

   Reconstitute in 220 µL Arginase Assay Buffer. Pipette up and down to dissolve completely. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.5 **Arginase Developer:**

   Reconstitute in 220 µL Arginase Assay Buffer. Pipette up and down to dissolve completely. Aliquot developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.
9.6 Arginase Converter Enzyme:
Reconstitute in 220 µL Arginase Assay Buffer. Pipette up and down to dissolve completely. Aliquot converter enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.7 H₂O₂ Standard:
Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.8 Arginase Positive Control:
Reconstitute in 100 µL Arginase Assay Buffer and dissolve completely. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within 2 months.
10. **STANDARD PREPARATION**

- Always prepare a fresh set of standards for every use.
- Diluted hydrogen peroxide is unstable. Discard unused diluted standard after use.

10.1 Prepare a 10 mM H$_2$O$_2$ standard by diluting 4 µL of the provided H$_2$O$_2$ standard with 348 µL of ddH$_2$O.

10.2 Prepare a 1 mM H$_2$O$_2$ standard by diluting 100 µL of the 10 mM H$_2$O$_2$ standard with 900 µL of ddH$_2$O.

10.3 Using the 1 mM H$_2$O$_2$ standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End [H$_2$O$_2$] in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0 nmol/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>2 nmol/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>4 nmol/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>6 nmol/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>8 nmol/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>10 nmol/well</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).
11. **SAMPLE PREPARATION**

**General Sample information:**

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 **Cell (adherent or suspension) samples:**

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = $1 \times 10^6$ cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold Assay Buffer on ice.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge sample for 5 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold Assay Buffer.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
11.2.5 Centrifuge samples for 5 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

**NOTE:** We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

High urea content in samples will interfere with the assay. To remove urea from samples, we suggest using a 10 kD Spin column (ab93349). In brief, add 50 – 200 µL of sample into a spin column. Centrifuge at 15,000 x g for 2 min. Replenish the lost liquid and repeat 2 times, **discarding** the filtrate, and bringing the sample (retentate) to its original volume with Arginase Assay Buffer.
12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Set up Reaction wells:
- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 40 µL samples (adjust volume to 40 µL/well with Assay Buffer).
- Positive control = 0.2 – 2 µL Positive control (adjust volume to 40 µL/well with Assay Buffer).
- Background control sample wells= 1 – 40 µL samples (adjust volume to 40 µL/well with Assay Buffer). **NOTE:** for samples with high urea content as it will interfere with the assay.

12.2 Substrate Mix:
Prepare 10 µL of Substrate Mix for samples. DO NOT PREPARE MIX TO STANDARD WELLS.

<table>
<thead>
<tr>
<th>Component</th>
<th>Substrate Mix (µL)</th>
<th>Background Control Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginase Assay Buffer</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Arginase Substrate</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

12.3 Add 10 µL of Substrate Mix to each sample and positive control. DO NOT ADD TO STANDARD WELLS.

12.4 Add 10 µL of Background Control Mix to each background control sample well

12.5 Mix and incubate for 20 minutes at 37°C.

12.6 Arginase Reaction Mix:
Prepare 50 µL of Reaction Mix for each reaction
Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

\[ X \, \mu L \, \text{component} \times (\text{Number samples} + \text{Standards} +1) \].

12.7 Add 50 µL of Reaction Mix into each standard, sample and Background control sample wells.

12.8 Measure absorbance immediately on a microplate reader at OD = 570 nm in kinetic mode for 10 – 30 minutes at 37°C.

**NOTE:** Sample Incubation time can vary depending on arginase activity. We recommend measuring the OD in kinetic mode, and choosing two time points \((T_1\) and \(T_2\)) in the linear range to calculate the arginase activity of the samples. The standard curve can be read in endpoint mode (i.e. at the end of the incubation time).
13. **CALCULATIONS**

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of arginase.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Activity of arginase is calculated as:

\[ \Delta OD = (OD_2 - OD_{BG2}) - (OD_1 - OD_{BG1}) \]

Where:

- \( OD_1 \) and \( OD_2 \) is the sample reading at time T1 and T2 respectively
- \( OD_{BG1} \) and \( OD_{BG2} \) is the sample background control reading at time T1 and T2 respectively

13.6 Use the \( \Delta OD \) to obtain B nmol of \( H_2O_2 \) generated by arginase.

13.7 Arginase activity (in nmol/min/µL or mU/µL) in the test samples is calculated as:

\[
Arginase \text{ Activity } = \left( \frac{B}{\Delta T \times V} \right) \times D
\]

\[ = \text{ nmol/min/} \mu\text{L} = \text{ mU/}\mu\text{L} = \text{ U/mL} \]
Where:

B = Amount of H$_2$O$_2$ from Standard Curve (nmol).

$\Delta T$ = Reaction time (minutes).

V = sample volume added into the reaction well (µL).

D = sample dilution factor.

**Unit Definition:**

1 Unit Arginase activity = amount of arginase that will generate 1.0 nmol of H$_2$O$_2$ per minute at pH 8 at 37°C.
14. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

![Figure 1](image1.png)

**Figure 1.** Typical H₂O₂ standard calibration curve using colorimetric reading.

![Figure 2](image2.png)

**Figure 2.** Arginase activity in rat liver lysate (3 µg) and positive control (2 µL).
15. **QUICK ASSAY PROCEDURE**

**NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare standard, substrate, enzyme mix, developer, converter enzyme and probe (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings), including deproteinization step.
- Set up plate for standard (50 μL), samples (40 μL) and background sample control wells (40 μL).
- Prepare 10 μL of Substrate Mix for each sample and positive control; and 10 μL of Background Control Mix for each background control sample well.

<table>
<thead>
<tr>
<th>Component</th>
<th>Substrate Mix (µL)</th>
<th>Background Control Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginase Assay Buffer</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Arginase Substrate</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

- Mix well and incubate for 20 min at 37°C.
- Prepare Arginase Reaction Mix (Number samples + background control sample + standards + positive control + 1).

<table>
<thead>
<tr>
<th>Component</th>
<th>Substrate Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginase Assay Buffer</td>
<td>42</td>
</tr>
<tr>
<td>Arginase Enzyme Mix</td>
<td>2</td>
</tr>
<tr>
<td>Arginase Developer</td>
<td>2</td>
</tr>
<tr>
<td>Arginase Converter Enzyme</td>
<td>2</td>
</tr>
<tr>
<td>OxiRed™ Probe</td>
<td>2</td>
</tr>
</tbody>
</table>

- Add 50 μL of Arginase Reaction Mix to the standard, sample, background control sample and positive control wells.
• Measure absorbance immediately on a microplate reader at OD=570 nm in kinetic mode, every 2 – 3 minutes, for 10 – 30 min at 37°C.
# Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different 96-well plate</td>
<td>Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate</td>
</tr>
<tr>
<td>Sample with erratic readings</td>
<td>Samples not deproteinized (if indicated on protocol)</td>
<td>Use PCA precipitation protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</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>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</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>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>--------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard readings do not follow a linear pattern</td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions on protocol</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear range</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. FAQ
18. **INTERFERENCES**

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- **RIPA:** contains SDS which can destroy/decrease the activity of the enzyme.
- **Urea.**
19. NOTES
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

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