

ab180877

Arginase Activity Assay Kit (Colorimetric)

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.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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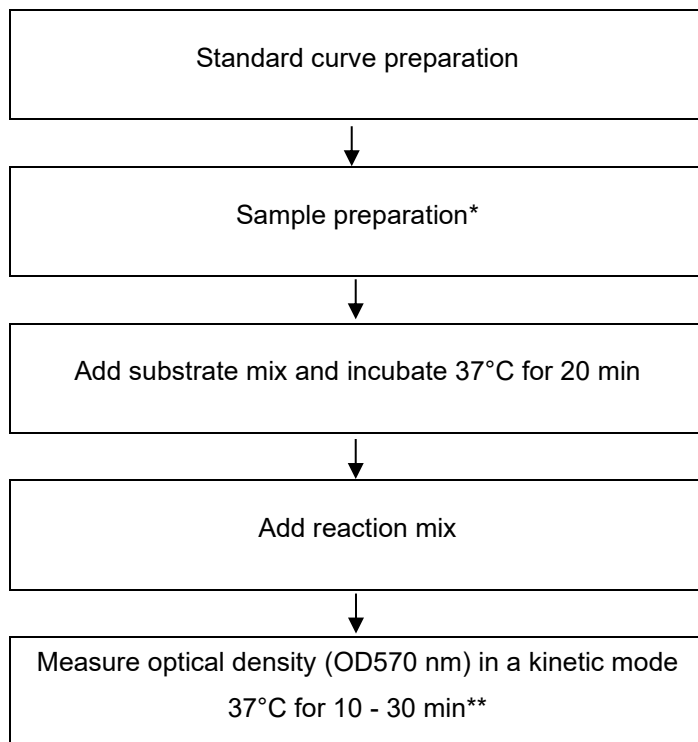
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1. **BACKGROUND**

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



*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 -20°C in the dark immediately upon receipt.

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

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer XIV/Arginase Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/OxiRed™ probe (in DMSO)	200 µL	-20°C	-20°C
Arginine/Arginase Substrate (lyophilized)	1 vial	-20°C	-20°C
Development Enzyme Mix I/Arginase Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Developer I/Arginase Developer (lyophilized)	1 vial	-20°C	-20°C
Converter Enzyme V/Arginase Converter Enzyme (lyophilized)	1 vial	-20°C	-20°C
H ₂ O ₂ Standard/H ₂ O ₂ Standard (0.88 M)	100 µL	-20°C	-20°C
Arginase Positive Control/Arginase Positive Control (lyophilized)	1 vial	-20°C	-20°C

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 **Assay Buffer XIV/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/OxiRed Probe – in DMSO:**

Ready to use as supplied. Warm the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.

For multiple use aliquot and store at -20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze–thaw cycles).

9.3 **Arginine/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 **Development Enzyme Mix I/Arginase Enzyme Mix:**

Reconstitute in 220 µL Assay Buffer XIV/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 **Developer I/Arginase Developer:**

Reconstitute in 220 µL Assay Buffer XIV/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 **Converter Enzyme V/Arginase Converter Enzyme:**

Reconstitute in 220 μL Assay Buffer XIV/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 Assay Buffer XIV/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 H₂O₂ standard by diluting 4 µL of the provided H₂O₂ standard with 348 µL of ddH₂O.

10.2 Prepare a 1 mM H₂O₂ standard by diluting 100 µL of the 10 mM H₂O₂ standard with 900 µL of ddH₂O.

10.3 Using the 1 mM H₂O₂ standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

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

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 = 1×10^6 cells).
- 11.1.2 Wash cells with cold PBS.
- 11.1.3 Resuspend cells in 100 μ L 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 μ L 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 Assay Buffer XIV/Arginase Assay Buffer.

12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and the prepared reagents Assay Buffer XIV/Arginase Assay Buffer 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 μ L standard dilutions.
- Sample wells = 1 – 40 μ L samples (adjust volume to 40 μ L/well with Assay Buffer).
- Positive control = 0.2 – 2 μ L Positive control (adjust volume to 40 μ L/well with Assay Buffer).
- Background control sample wells= 1 – 40 μ L samples (adjust volume to 40 μ L/well with Assay Buffer). **NOTE:** for samples with high urea content as it will interfere with the assay.

12.2 Substrate Mix:

Prepare 10 μ L of Substrate Mix for samples. DO NOT PREPARE MIX TO STANDARD WELLS.

Component	Substrate Mix (μ L)	Background Control Mix (μ L)
Assay Buffer XIV/Arginase Assay Buffer	8	10
Arginine/Arginase Substrate	2	0

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

12.4 Add 10 μ L 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 μ L of Reaction Mix for each reaction

ASSAY PROCEDURE and DETECTION

Component	Reaction Mix (μL)
Assay Buffer XIV/Arginase Assay Buffer	42
Development Enzyme Mix I/Arginase Enzyme Mix	2
Developer I/Arginase Developer	2
Converter Enzyme V/Arginase Converter Enzyme	2
OxiRed Probe	2

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:

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

- 12.7 Add 50 μL of Reaction Mix into each standard, test sample, Background control sample and positive control 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:

OD₁ and OD₂ 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 ΔOD to obtain B nmol of H₂O₂ generated by arginase.

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

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

$$= \text{nmol/min/} \mu\text{L} = \text{mU/} \mu\text{L} = \text{U/mL}$$

Where:

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

ΔT = 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 μmol of H_2O_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.

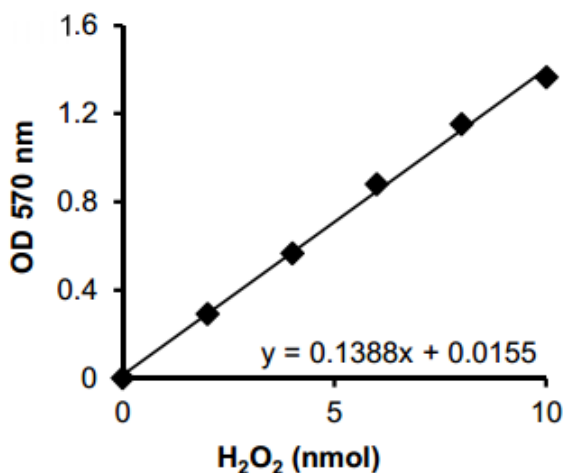


Figure 1. Typical H_2O_2 standard calibration curve using colorimetric reading.

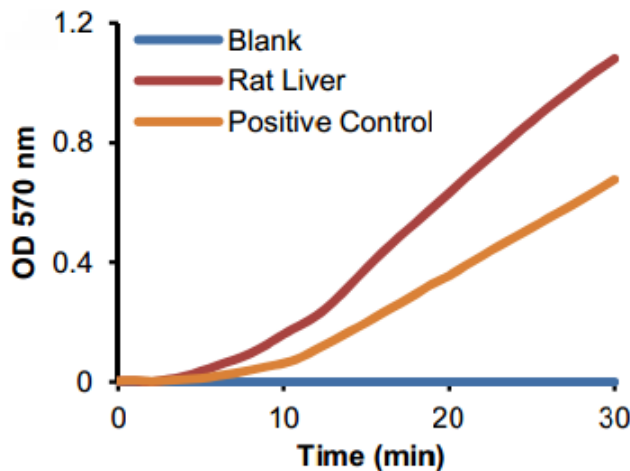


Figure 2. Arginase activity in rat liver lysate (3 μg) and positive control (2 μL).

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 μ L), samples (40 μ L) and background sample control wells (40 μ L).
- Prepare 10 μ L of Substrate Mix for each sample and positive control; and 10 μ L of Background Control Mix for each background control sample well.

Component	Substrate Mix (μ L)	Background Control Mix (μ L)
Assay Buffer XIV/Arginase Assay Buffer	8	10
Arginine/Arginase Substrate	2	0

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

Component	Substrate Mix (μ L)
Assay Buffer XIV/Arginase Assay Buffer	42
Development Enzyme Mix I/Arginase Enzyme Mix	2
Developer I/Arginase Developer	2
Converter Enzyme V/Arginase Converter Enzyme	2
OxiRed Probe/OxiRed™ Probe	2

RESOURCES

- 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.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

RESOURCES

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

Can catalase in the tissue lysates interfere with the assay by consuming the H₂O₂?

Endogenous enzymes in the samples, like catalase, can interfere with the assay. However, it will boil down to the relative abundance of catalase vs arginase and also the individual K_m values. According to our data, we could detect Arginase activity in liver samples higher than the background.

Can you test serum samples?

There is not much Arginase enzyme in serum under normal conditions. Arginase 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. For testing serum samples, 1-40ul can be tested directly per well without any preparation.

Can we use frozen samples with this assay?

We would recommend using fresh samples. However, frozen samples can be used too provided they have not undergone multiple freeze thaw cycles.

Can the kit work on bacteria or yeast cells?

The kit has been standardized for mammalian cells only.

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

Technical Support

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For all technical or commercial enquiries please go to:

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<https://www.abcam.cn/contact-us> (China)

<https://www.abcam.co.jp/contact-us> (Japan)