

Version 5a, Last updated 16 July 2025

ab241018

Albumin Creatinine Ratio

Assay Kit

For the Estimation of albumin/creatinine and the ACR in biological samples.

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

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1. Overview

The Albumin Creatinine Ratio Assay Kit (ab241018) provides a simple, sensitive, and high throughput adaptable assay that detects albumin (detection range: 0.02- 2.5 mg/mL), creatinine (detection range: 0.002 -0.5 mg/mL) and Albumin-to-creatinine ratio.

The ACR ratio is determined in two steps: First, albumin is determined by using a probe (AB580) that specifically recognizes albumin (Ex/Em = 600/630 nm). Second, creatinine is converted to sarcosine via enzymatic reactions. Sarcosine is specifically oxidized generating a product that reacts with a probe producing a chromophore that can be detected at 570 nm.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.



Prepare the Creatine standard curve using the 10 $\mu\text{g}/\mu\text{L}$ Creatine standard. Dilute to 0.1 $\mu\text{g}/\mu\text{L}$ using dH_2O .



Prepare the BSA standard curve using the 2 mg/mL BSA Standard II. Dilute to 0.5 mg/mL using Albumin diluent.



Create the Reaction Mix, add 50 μl to each well.



Create the Background Control Mix and add 50 μl to wells containing sample background control.



Incubate the plate at 37°C for 1 h. Measure absorbance at 570 nm in end-point mode.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Briefly centrifuge small vials prior to opening.

Item	Quantity	Storage condition
Assay Buffer 34	25 mL	-20°C
Albumin Assay Buffer II	7 mL	-20°C
Albumin Diluent	7 mL	-20°C
Albumin Probe	400 µL	-20°C
OxiRed™ Probe	0.2 mL	-20°C
Creatinase	1 vial	-20°C
Creatininase	1 vial	-20°C
Sarcosine Enzyme Mix	1 vial	-20°C
BSA Standard II	1 ml	-20°C or Ambient
Creatine Standard	1 vial	-20°C

PLEASE NOTE: Assay Buffer 34 was previously labelled as Assay Buffer XXXIV and Creatine Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Creatinine Probe. The composition has not changed.

5. Materials Required, Not Supplied

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

- 96-well white plate with flat bottom
- 96-well clear plate with flat bottom
- 10 kDa Spin Column
- Multi-well spectrophotometer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Assay Buffer 34:

Store at -20 °C. Bring to room temperature before use.

6.2 Albumin Assay Buffer II:

Store at -20 °C. Bring to room temperature before use.

6.3 Albumin Diluent:

Store at -20 °C. Bring to room temperature before use.

6.4 Albumin Probe:

Light sensitive. Store at -20°C. Bring to room temperature before use.

6.5 OxiRed™ Probe:

Light sensitive. Store at -20°C. Bring to room temperature before use.

6.6 Creatinase:

Reconstitute with 220 µL of Assay Buffer 34. Aliquot and store at -20°C. Freeze/Thaw should be limited to one time. Keep on ice during use.

6.7 Creatininase:

Reconstitute with 220 µL of Assay Buffer 34. Aliquot and store at -20°C. Freeze/Thaw should be limited to one time. Keep on ice during use.

6.8 Sarcosine Enzyme Mix:

Reconstitute with 220 µL of Assay Buffer 34. Aliquot and store at -20°C. Freeze/Thaw should be limited to one time. Keep on ice during use.

6.9 BSA Standard II:

Store at room temperature.

Creatinine:

Constitute with 115 µL of dH₂O to generate 10 µg/µL Creatinine Standard. Dissolve completely. Store at -20°C.

Use within 2 months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 BSA Standard curve

- 7.1.1 Dilute the 2 mg/mL BSA Standard II to 0.5 mg/mL by adding 25 μ L of the standard to 75 μ L of Albumin diluent.
- 7.1.2 Add 0, 2, 4, 6, 8, and 10 μ L of the 0.5 mg/mL BSA Standard II to wells of the 96 well plate.
- 7.1.3 Bring the total volume of each well to 50 μ L with Albumin Diluent to generate 0, 1, 2, 3, 4, 5 μ g of BSA Standard II per well.

Standard #	0.5 mg/mL BSA Standard II (μL)	Albumin Diluent (μL)	BSA Standard II Per well (μg)
1	10	40	5
2	8	42	4
3	6	44	3
4	4	46	2
5	2	48	1
6	0	50	0

7.2 Creatinine Standard Curve:

- 7.2.1 Dilute the 10 µg/mL Creatinine standard to 0.1 µg/µL by adding 10 µL of the standard to 990 µL of ddH₂O.
- 7.2.2 Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 µg/µL Creatinine standard to wells of the 96 well plate.
- 7.2.3 Bring the total volume of each well to 50 µL with Assay Buffer 34 to generate 0, 0.2, 0.4, 0.6, 0.8, and 1 µg of Creatinine standard per well.

Standard #	0.1 µg/µL Creatinine Standard (µL)	Assay Buffer 34 (µL)	Creatinine Per well (µg)
1	10	40	1
2	8	42	0.8
3	6	44	0.6
4	4	46	0.4
5	2	48	0.2
6	0	50	0

8. Sample Preparation

8.1 Albumin Assay:

- Centrifuge urine sample at 4000 x g, 4°C for 3 min., if precipitation is observed. Collect supernatant.
- Add 1-50 µL into desired well(s) in a 96-well white plate. Adjust the volume to 50 µL/well with Albumin Diluent.

ΔNote: For saliva samples, centrifuge sample at 10,000 x g, 4°C for 10 min., if precipitation is observed. Collect supernatant. Add 1-50 µL into desired well(s) in a 96-well white plate. Adjust the volume to 50 µL/well with Albumin Diluent.

ΔNote: Metabolites found in biological samples do not contribute significantly to the background signal. However, if interference is observed in the sample, prepare parallel sample well(s) as sample background control(s). Make up the volume to 50 µL/well with Albumin Diluent.

ΔNote: Albuminuria concentration is over a wide range depending on the sample. Albumin concentration in human urine (mg Albumin/L) is - normal: < 10; microalbuminuria: 20 – 200; and macroalbuminuria > 200. For unknown samples, we recommend doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.

8.2 Creatinine Assay Protocol:

- Centrifuge urine sample at 4000 x g, 4°C for 3 min, if precipitation is observed. Collect supernatant.
- Add 2-50 µL into desired well(s) in a 96-well clear plate. Adjust the volume to 50 µL/well with Assay Buffer 34.

ΔNote: For samples having medium and high concentrations of protein such as serum, deproteinize sample using a 10 kDa spin column. Briefly, centrifuge at 10,000 x g, 4°C for 10 min. Collect the filtrate. Add 2-50 µL into desired well(s) in a 96-well clear plate and adjust the volume to 50 µL/well with Assay Buffer 34.

ΔNote: Creatinine concentration varies over a wide range depending on the sample. For unknown samples, we recommend doing pilot experiment and testing different dilutions using Assay Buffer 34 (1:50 – 1:200) to ensure the readings are within the Standard Curve range.

ΔNote: Endogenous compounds such as sarcosine and creatine may interfere with the assay. We recommend preparing parallel well(s) as sample background control.

9. Albumin Assay Procedure

- 9.1 Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 μ L Mix containing the following components. Mix well before use:

	Reaction Mix	Background Mix
Albumin Assay Buffer II	46 μ L	50 μ L
Albumin Probe	4 μ L	/

- 9.2** Add 50 μ L of the Reaction Mix to each well containing the BSA Standard II (s), Sample(s); Add 50 μ L of Background Mix to well(s) containing Sample Background Control.
- 9.3 Measurement:** Incubate plate at 25°C for 30 min, protected from light. Measure fluorescence (Ex/Em = 600/630 nm) in end point mode.

10. Creatinine Assay Protocol

10.1 Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 μL Mix containing the following components. Mix well before use:

	Reaction Mix	Background Mix
Assay Buffer 34	42 μL	44 μL
Creatinase	2 μL	2 μL
Creatininase	2 μL	/
Sarcosine Enzyme Mix	2 μL	2 μL
OxiRed™ Probe	2 μL	2 μL

10.2 Add 50 μL of the Reaction Mix to each well containing the Creatinine Standard(s), Sample(s); Add 50 μL of Background Mix to well(s) containing Sample Background Control.

10.3 Measurement: Incubate plate at 37°C for 60 min, protected from light. Measure absorbance (OD 570 nm) in end point mode.

11. Data Analysis

11.1 Albumin assay

- 11.1.1 Subtract the 0 BSA Standard reading from all Standard curve readings. Plot the background-subtracted BSA Standard Curve and calculate the slope.
- 11.1.2 If sample background control slope is significant, then subtract sample background control reading from sample readings.
- 11.1.3 Apply the corrected Δ RFU value to the BSA Standard Curve to get B μ g Albumin in the sample well.

$$\text{Sample Albumin Concentration} = (B/V) \times D \mu\text{g}/\mu\text{L} = \text{mg/ml}$$

Where:

B is the amount of Albumin in the sample well from Standard Curve (μ g)

V is the sample volume added into the reaction well (μ L)

D is the sample dilution factor

BSA molecular weight \approx 66.5 kDa

1 mg/ml \equiv 1 μ g/ μ L \equiv 1000 mg/L \equiv 100 mg/dl

11.2 Creatinine assay

- 11.2.1 Subtract the 0 Creatinine Standard reading from all Standard curve readings. Plot the background-subtracted Creatinine Standard Curve and calculate the slope.
- 11.2.2 If sample background control slope is significant, then subtract sample background control reading from sample readings.
- 11.2.3 Apply the corrected OD value to the Creatinine Standard Curve to get B μg Creatinine in the sample well.

$$\text{Sample Creatinine Concentration} = (B/V) \times D \mu\text{g}/\mu\text{L} = \text{mg/ml}$$

Where:

B is the amount of Albumin in the sample well from Standard Curve (μg)

V is the sample volume added into the reaction well (μL)

D is the sample dilution factor

Creatinine molecular weight: 113.12 g/mol

1 mM Creatinine \equiv 1 nmol Creatinine/ μL \equiv 0.113 mg/ml \equiv 0.0113 mg/dl

Estimation of Albumin-to-Creatinine Ratio (ACR)

Estimate ACR by using albumin and creatinine concentrations established in sample(s) using the formula:

$$\frac{\text{Albumin} \left(\frac{\text{mg}}{\text{dL}} \right)}{\text{Creatinine} \left(\frac{\text{g}}{\text{dL}} \right)} = \text{ACR} \frac{\text{mg Albumin}}{\text{g Creatinine}} \approx \frac{\text{Excreted Albumin (mg)}}{24 \text{ hr}}$$

ΔNote: Albuminuria and Albumin-to-Creatinine Ratio (in mg Albumin/g Creatinine) have been defined as follows: Normal: $0 \leq \text{ACR} \leq 30$; Microalbuminuria: $30 \leq \text{ACR} \leq 300$; Proteinuria Clinical: $\text{ACR} > 300$.

ΔNote: Chronic Kidney Disease (CKD) may be present if $\text{ACR} \geq 30$.

12. Typical Data

Typical data provided for demonstration purposes only.

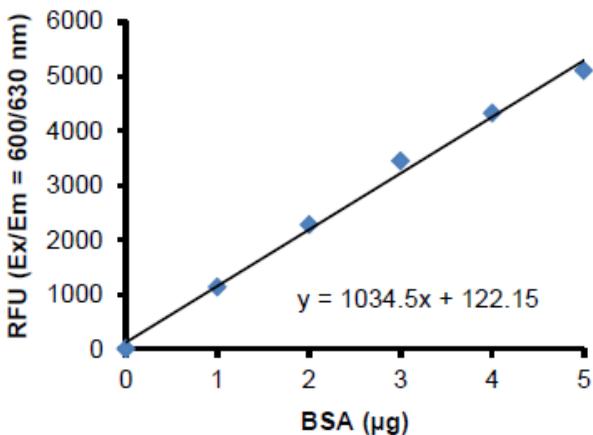


Figure 1. BSA Standard Curve.

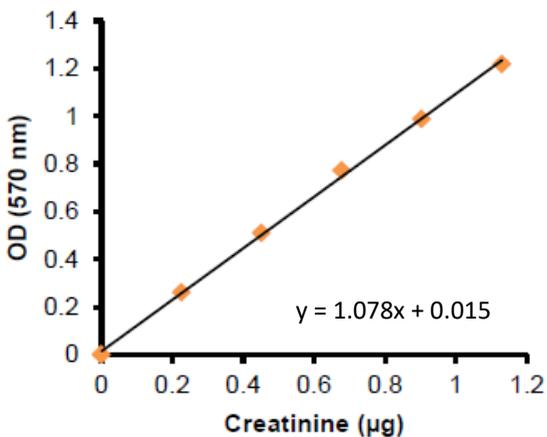


Figure 2: Creatinine standard curve.

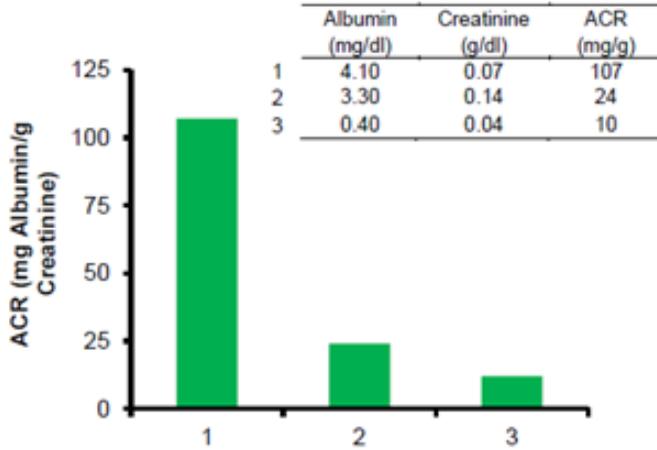


Figure 3: Estimation of ACR in human urine in diabetic (1) and non-diabetic donors (2, and 3). For Albumin, 50 µl of undiluted samples and for reatinine, 30 µl of diluted samples (100 times diluted using Assay Buffer 34) were assayed following the kit protocol.

13. Notes

Technical Support

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