

Version 1 Last updated 9 May 2023

# ab241017

## Albumin Assay Kit (Fluorometric)

For the measurement of Albumin in biological fluids from various mammalian 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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## 1. Overview

The Albumin Assay Kit (Fluorometric) (ab241017) provides a simple, sensitive, and high-throughput adaptable assay that detects albumin concentrations in healthy and Albuminuric urine samples.

This assay is based on the fluorometric detection of albumin using a probe (AB580) that specifically recognizes albumin (Ex/Em: 600/630 nm). AB580 does not cause fluorescence in presence of other proteins or metabolites such as IgG, urea, ascorbic acid and glucose. Our assay can detect albumin concentrations as low as 20 mg/L.

## 2. Protocol Summary

Prepare all samples, controls and standards as instructed.



Prepare the standard curve using the 2 mg/mL BSA standard. Dilute to 0.5 mg/mL using Albumin Diluent.



Create the Reaction Mix, add 50  $\mu$ l to each well.



Incubate the plate at 25°C for 30 minutes, protected from the light.  
Measure the fluorescence at (Ex/Em: 600/630 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](http://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

- Store kit at 4°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if 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
Albumin Assay Buffer II/Albumin Assay Buffer	7 mL	4°C
Albumin Diluent	7 vial	4°C
Albumin Probe	0.4 mL	4°C
BSA Standard II/BSA standard (2 mg/mL)	1 mL	4°C

## 5. Materials Required, Not Supplied

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

- Multi-well spectrophotometer (ELISA plate reader).
- 96-well white plate with flat bottom.

## 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 Albumin Assay Buffer II/Albumin Assay Buffer:

Store at either 4 °C. Bring to room temperature before use.  
Ready to use as supplied.

### 6.2 Albumin Diluent:

Store at either 4 °C. Bring to room temperature before use.  
Ready to use as supplied.

### 6.3 Albumin Probe:

Light sensitive. Ready to use as supplied. Warm to RT before use. Store at 4°C. Use within two months.

### 6.4 BSA Standard II/BSA Standard:

Ready to use as supplied. Bring to RT before use. Store at 4 °C.  
Use within two 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 Prepare 0.5 mg/mL BSA standard by adding 25  $\mu$ L of 2 mg/mL BSA standard into 75  $\mu$ L of Albumin Diluent.
- 7.2 Add 0, 2, 4, 6, 8, and 10  $\mu$ L of the 0.5 mg/mL BSA Standard II /BSA standard into the wells of the 96 well plate to generate 0, 1, 2, 3, 4 and 5  $\mu$ g of BSA per well.
- 7.3 Bring the total volume of each well to 50  $\mu$ L with Albumin Diluent.

Standard #	0.5 mg/mL BSA standard ( $\mu$ L)	Albumin Diluent ( $\mu$ L)	BSA Per well ( $\mu$ g/well)
1	10	40	5
2	8	42	4
3	6	44	3
4	4	46	2
5	2	48	1
6	0	50	0

## 8. Sample Preparation

### 8.1 Urine samples:

- Centrifuge samples at 4,000 x g, 3 min., 4°C, if precipitation is observed. Collect supernatant
- Add 1-50 µL sample into desired well(s) in a 96-well plate.
- Adjust the volume to 50 µL/well with Albumin Diluent.

### 8.2 Saliva Samples:

- Centrifuge samples at 10,000 x g, 10 min., 4°C. Collect supernatant.
- Add 1-50 µL sample into desired well(s) in a 96-well 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 varies over a wide range depending on the patient's medical history (See table below). For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve linear range.

	24 hrs Urine (mg/24 hrs)	Albumin (mg/mL)
Normal	<15	<10
High Normal	15 – 30	10 - 20
Microalbuminuria	30 – 300	20 - 200
Macroalbuminuria	>300	>200

## 9. Assay Procedure

- 9.1 Reaction Mix:** Mix enough reagents for the total number of wells to be assayed including Standards and samples. For each well, prepare 50  $\mu\text{L}$  of Reaction Mix containing:

	Reaction Mix	Background Control
Albumin Assay Buffer II/Albumin Assay Buffer	46 $\mu\text{L}$	50 $\mu\text{L}$
Albumin Probe	4 $\mu\text{L}$	/

- 9.2** Mix well. Add 50  $\mu\text{L}$  of Reaction Mix into each well. Mix well.
- 9.3 Measurement:** Incubate plate at 25°C for 30 min., protected from light. Measure fluorescence (Ex/Em: 600/630 nm) with end point setting.

## 10. Data Analysis

- 10.1 Subtract the 0 BSA Standard reading from all Standard curve readings. Plot the background-subtracted BSA Standard Curve and calculate the slope.
- 10.2 If sample background control slope is significant, then subtract sample background control reading from sample readings.
- 10.3 Apply the corrected  $\Delta$ RFU value to the BSA Standard Curve to get B  $\mu$ g of 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 ( $\mu$ g)

**V** is the sample volume added into the reaction well ( $\mu$ L)

**D** is the sample dilution factor

BSA molecular weight  $\approx$  66.5 kDa

1 mg/ml  $\equiv$  1  $\mu$ g/ $\mu$ L  $\equiv$  1000 mg/L  $\equiv$  0.1 g/dl

Albumin concentration can also be expressed as  $\mu$ g Albumin/min or mg Albumin/mg Creatinine.

## 11. Typical Data

Typical data provided for demonstration purposes only.

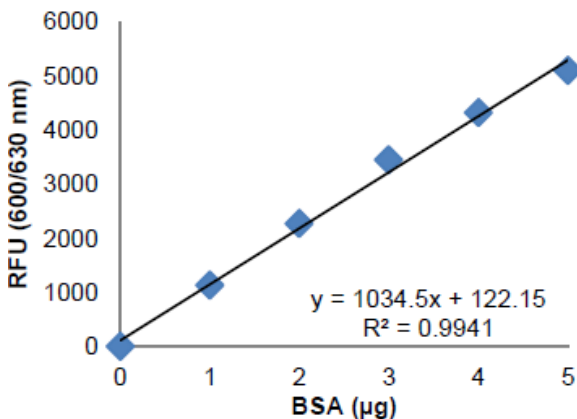


Figure 1. BSA Standard Curve.

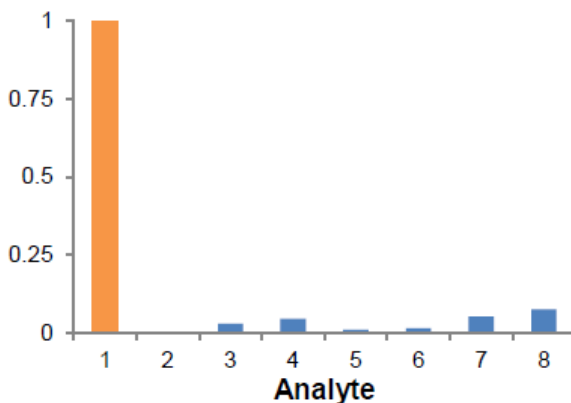
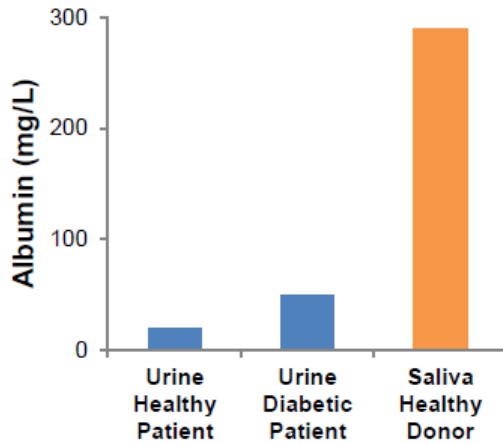


Figure 2. Relative fluorescence of selected metabolites and proteins found in human urine. 1) Human Serum Albumin (30 µg) was assayed and compared with 2) Ascorbic Acid (20 mM), 3) Glucose (20 mM), 4) Creatinine (20 mM), and 5) Urea (20 mM), 6) Lysozyme (30 µg), 7) Trypsin (30 µg), and 8) IgG (30 µg).



**Figure 3:** Measurement of Albumin in urine from healthy and diabetic donors (40  $\mu$ L each), and saliva (30  $\mu$ L). Samples were assayed following kit protocol. Albumin concentrations (in mg/L) are: healthy donor urine: 19.4; diabetic donor urine: 49.0; Saliva: 290.

## 12. Notes



# Technical Support

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