Albumin (Albuminuria) Fluorometric Assay Kit (#BN00780)

(Catalog # BN00780; 100 assays; Store at 4°C)

I. Introduction:

Albuminuria is a pathological condition characterized by the presence of high concentrations of albumin in urine. Albumin concentration ranges between 30-300 mg/L in the patient's urine sample, which is not detected by commercially available protein tests. Albuminuria has become a useful marker for the early detection of chronic kidney diseases in patients suffering from diabetes, hypertension, and cardiovascular diseases. Assay Genie's Albumin (Albuminuria) Assay Kit 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.

II. Application:

- Measurement of Albumin in biological samples from different mammalian species

III. Sample Type:

- Biological fluids: Urine, Saliva

IV. Kit Contents:

Components	BN00780	Cap Code	Part Number
Albumin Assay Buffer	7 ml	NM	BN00780-1
Albumin Diluent	7 ml	NM/Blue	BN00780-2
Albumin Probe (AB580)	0.4 ml	Brown	BN00780-3
BSA Standard (2 mg/ml)	1 ml	White	BN00780-4

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **Albumin Assay Buffer and Albumin Diluent**: Bring to room temperature (RT) before use. Store at 4°C.
- **Albumin Probe (AB580):** Light sensitive. Ready to use as supplied. Warm to RT before use. Store at 4°C. Use within two months.
- **BSA Standard (2 mg/ml):** Ready to use as supplied. Bring to RT before use. Store at 4 °C. Use within two months.

VII. Albumin/Albuminuria Assay Protocol:

1. **Sample Preparation:** For urine: Centrifuge samples at 4,000 x g, 3 min., 4°C, if precipitation is observed. Collect supernatant. For saliva: 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.

   Notes:

   a. 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.

   b. 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 h Urine (mg/24 h)	Albumin (mg/L)
Normal	< 15	< 10
High Normal	15 - 30	10 - 20
Microalbuminuria	30 - 300	20 - 200
Macroalbuminuria	> 300	> 200

2. **Standard Curve Preparation:** Prepare 0.5 mg/ml BSA Standard by adding 25 µl of 2 mg/ml BSA Standard into 75 µl of Albumin Diluent. Add 0, 2, 4, 6, 8, and 10 µl of 0.5 mg/ml BSA Standard into a series of wells in a 96-well plate to generate 0, 1, 2, 3, 4 and 5 µg of BSA/well. Adjust the volume to 50 µl/well with Albumin Diluent.
3. **Reaction Mix:** Mix enough reagents for the total number of wells to be assayed including Standards and samples. For each well, prepare 50 µl of Reaction Mix containing:

	Reaction Mix	Background Control
Albumin Assay Buffer	46 µl	50 µl
Albumin Probe (AB580)	4 µl	----

Mix well. Add 50 µl of Reaction Mix into each well. Mix.

4. **Measurement:** Incubate plate at 25°C for 30 min., protected from light. Measure fluorescence (Ex/Em: 600/630 nm) with end point setting.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the BSA Standard Curve. If sample background (i.e. Urine + Albumin Assay Buffer) control is significant, then subtract sample background control reading from sample readings. Apply corrected RFU to BSA Standard Curve to get B µg of Albumin in the sample wells.

Sample Albumin Concentration (C) = B/V X D µg/µl or mg/ml

Where: **B** is amount of Albumin from Standard Curve (µg)
V is sample volume added into the reaction well (µl)
D is sample dilution factor
BSA molecular weight ≈ 66.5 kDa
1 mg/ml ≡ 1 µg/µl ≡ 1000 mg/L ≡ 0.1 g/dl

Albumin concentration can also be expressed as µg Albumin/min or mg Albumin/mg Creatinine.

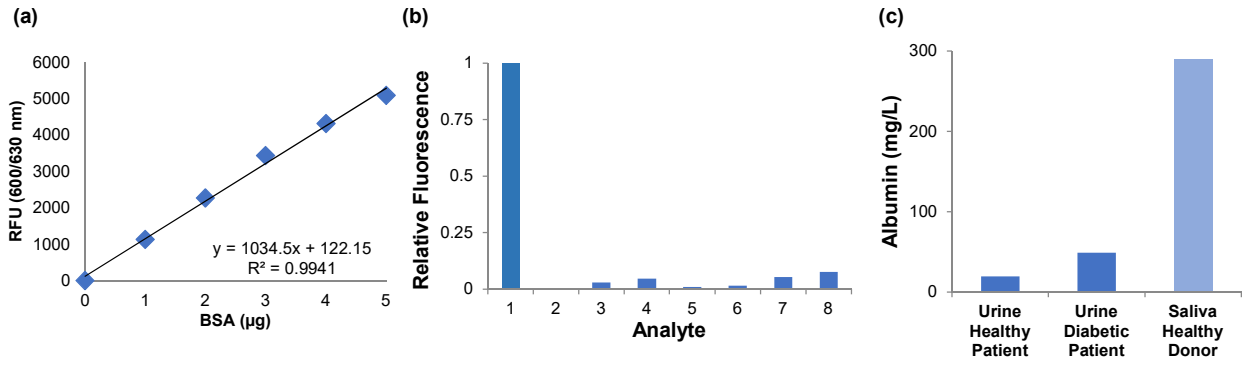

Figure: (a) BSA Standard Curve. (b) Relative fluorescence of selected metabolites and proteins found in human urine. 1) Human Serum Albumin (30 µg) was assayed and compared with 2) Ascorbic Acid (20 mM), 3) Glucose (20 mM), 4) Creatinine (20 mM), and 5) Urea (20 mM), 6) Lysozyme (30 µg), 7) Trypsin (30 µg), and 8) IgG (30 µg). (c) Measurement of Albumin in urine from healthy and diabetic donors (40 µl each), and saliva (30 µl). Samples were assayed following kit protocol. Albumin concentrations (in mg/L) are: healthy donor urine: 19.4; diabetic donor urine: 49.0; Saliva: 290.

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