Albumin-to-Creatinine Ratio (ACR) Assay Kit (BN00781)

(Catalog # BN00781; 100 assays; Store at -20°C)

I. Introduction:

Albumin-to-Creatinine Ratio (ACR) is one of the two markers used to determine chronic kidney disease (CKD). ACR is recommended to be measured on regular basis on people living with Type I and Type II diabetes. ACR is defined as the ratio between albumin (reported in mg/dl) and creatinine (reported in g/dl). This ratio estimates the amount of albumin excreted in urine during a 24 hr period. Albuminuria is diagnosed when ACR is greater than 30 mg albumin/g creatinine. Assay Genie's ACR Assay Kit 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.

Step 1 Albumin $\xrightarrow{\text{Probe (AB580)}}$ Fluorescence (Ex/Em = 600/630 nm)

Step 2 Creatinine $\xrightarrow{\text{Creatininase}}$ Creatine $\xrightarrow{\text{Creatinase}}$ Sarcosine $\xrightarrow{\text{Oxidation}}$ Absorbance (OD 570 nm)

II. Application:

- Estimation of albumin in biological samples
- Estimation of creatinine in biological samples
- Determination of ACR in mammalian urine samples

III. Sample Type:

- Albumin: urine, saliva, etc.
- Creatinine: urine, serum, etc.
- ACR: urine

IV. Kit Contents:

Components	BN00781	Cap Code	Part Number
Creatinine Assay Buffer	25 ml	WM	BN00781-1
Albumin Assay Buffer	7 ml	NM	BN00781-2
Albumin Diluent	7 ml	Blue	BN00781-3
Albumin Probe (AB580)	0.4 ml	Brown	BN00781-4
Creatinine Probe	0.2 ml	Red	BN00781-5
Creatinase	1 vial	Blue	BN00781-6
Creatininase	1 vial	Purple	BN00781-7
Creatinine Enzyme Mix	1 vial	Green	BN00781-8
BSA Standard (2 mg/ml)	1 ml	White	BN00781-9
Creatinine Standard (10 µmol)	1 vial	Yellow	BN00781-10

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **Creatinine Assay Buffer, Albumin Assay Buffer, and Albumin Diluent**: Store at -20°C. Bring to room temperature (RT) before use.
- **Albumin Probe (AB580) and Creatinine Probe:** Light sensitive. Store at -20°C. Bring to RT before use.
- **Creatinase, Creatininase, and Creatinine Enzyme Mix:** Reconstitute with 220 µl of Creatinine Assay Buffer. Aliquot and store at -20°C. Freeze/thaw should be limited to one time. Keep on ice during use.
- **BSA Standard (2 mg/ml):** Store at RT.
- **Creatinine (10 µmol):** Reconstitute with 115 µl of dH_2O to generate 10 µg/µl Creatinine Standard. Dissolve completely. Store at -20°C. Use within 2 months.

VII. Albumin Assay Protocol:

1. **Sample Preparation:** 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.

   Notes:

   a. 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.

b. 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.

c. 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.

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 white plate to generate 0, 1, 2, 3, 4 and 5 µg of BSA Standard/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. For each well, prepare 50 µl of Reaction Mix containing:

	Reaction Mix	*Background Control Mix
Albumin Assay Buffer	46 µl	50 µl
Albumin Probe	4 µl	----

Mix well. Add 50 µl of Reaction Mix into Standard and sample wells. Mix.
* For samples having background, add Background Control Mix to background control well(s) and mix.

4. **Measurement:** Incubate plate at 25°C for 30 min., protected from light. Measure fluorescence (Ex/Em = 600/630 nm) in end point mode.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the BSA Standard Curve. If sample background control is significant, then subtract sample background control reading from sample reading. Apply sample's corrected RFU to Standard Curve to get B µg of Albumin in the sample well.

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

Where: **B** is amount of Albumin in the sample well 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 ≡ 100 mg/dl

VIII. Creatinine Assay Protocol:

1. **Sample Preparation:** 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 Creatinine Assay Buffer.

Notes:

a. 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 Creatinine Assay Buffer.

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

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

2. **Standard Curve Preparation:** Prepare 0.1 µg/µl Creatinine Standard by adding 10 µl of 10 µg/µl Creatinine Standard into 990 µl of ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 0.1 µg/µl Creatinine Standard into a series of wells in a 96-well clear plate to generate 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg of Creatinine/well. Adjust the volume to 50 µl/well with Creatinine Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the total number of wells to be assayed. For each well, prepare 50 µl of Reaction Mix containing:

	Reaction Mix	Background Control Mix*
Creatinine Assay Buffer	42 µl	44 µl
Creatinase	2 µl	2 µl
Creatininase	2 µl	---
Creatinine Enzyme Mix	2 µl	2 µl
Creatinine Probe	2 µl	2 µl

Mix well. Add 50 µl of Reaction Mix into Standard and sample wells. Mix.
* For samples having background, add Background Control Mix to background control well(s) and mix.

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

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Creatinine Standard Curve. If sample background control is significant, then subtract sample background control reading from sample reading. Apply sample's corrected OD to Standard Curve to get B nmol of Creatinine in the sample well.

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

Where: **B** is amount of Creatinine in the sample well from Standard Curve (µg)
V is sample volume added into the reaction well (µl)

D is sample dilution factor

Creatinine molecular weight: 113.12 g/mol

1 mM Creatinine ≡ 1 nmol Creatinine/µl ≡ 0.113 mg/ml ≡ 0.0113 mg/dl

IX. Estimation of Albumin-to-Creatinine Ratio (ACR):

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

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

Notes:

a. Albuminuria and Albumin-to-Creatinine Ratio (in mg Albumin/g Creatinine) have been defined as follows: Normal: 0 ≤ ACR ≤ 30; Microalbuminuria: 30 ≤ ACR ≤ 300; Proteinuria Clinical: ACR > 300.

b. Chronic Kidney Disease (CKD) may be present if ACR ≥ 30.

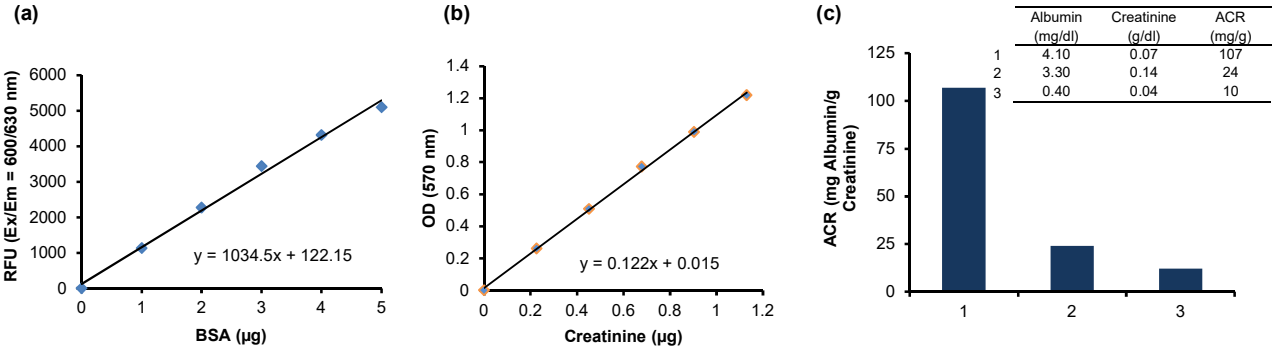

Figure: (a) BSA Standard Curve. **(b)** Creatinine Standard Curve. **(c)** Estimation of ACR in human urine in diabetic (1) and non-diabetic donors (2, and 3). For Albumin, 50 µl of undiluted samples and for Creatinine, 30 µl of diluted samples (100 times diluted using Creatinine Assay Buffer) were assayed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

FOR RESEARCH USE ONLY! Not to be used on humans.