Cytidine Deaminase Activity Assay Kit (Fluorometric) (#BN00693)

(Catalog # BN00693; 100 assays; Store at -20°C)

I. Introduction:

Cytidine Deaminase (3.5.4.5) is a homotetramer enzyme that catalyzes the irreversible deamination of cytidine to produce uridine, thus maintaining the intracellular pyrimidine pool of uridine. Cytidine as well as adenosine, thymidine, guanosine and uridine are one of the five standard nucleosides with which nucleic acids are composed. Nucleosides accumulate or are recycled from salvage pathways within the cell and play an important role in DNA and RNA synthesis and subsequent cell division. Tumorigenesis is intricately linked to metabolism of macromolecular precursors, specifically, the building blocks of DNA and RNA. Thus tumor cells have greater concentrations of uridine and cytidine analogues (402 µM +/- 252) compared to normal cells (83 µM +/- 133), to support the increased demand for nucleotides in mitosis in actively dividing cells. Assay Genie's Cytidine Deaminase Activity Assay Kit uses CDA to convert cytidine to the uridine and NH_3, as intermediates. The intermediate products then react with a proprietary Reaction Mix to generate a stable fluorophore that can be detected fluorometrically (Ex/Em= 410/470 nm). The kit is suitable for measuring CDA activity as low as 1 µUnit (1 pmole/min/well) in biological samples.

$$\text{Cytidine (substrate)} + H_2O \xrightarrow{\text{CDA}} \text{Uridine} + NH_3$$

$$NH_3 + \text{Reaction Mix} \longrightarrow \text{Stable Fluorophore (Ex/Em= 410/470 nm)}$$

II. Applications:

- Measurement of CDA activity in various tissues/cells
- Evaluation of CDA activity for comparison of activity rates in normal versus tumor tissues

III. Sample Type:

- Animal tissues: mouse or rat spleen, liver, and kidney.

IV. Kit Contents:

Components	BN00693	Cap Code	Part Number
CDA Assay Buffer	25 ml	WM	BN00693-1
CDA Substrate	1 vial	Red	BN00693-2
Cytidine Deaminase (CDA)	1 vial	Green	BN00693-3
Developer A	1.2 ml	Amber	BN00693-4
Ammonium Chloride Standard	0.1 ml	Yellow	BN00693-5
Microplate Sealing Film	1 film	---	BN00693-6

V. User Supplied Reagents and Equipment:

- 96-well white microplate plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- 2-Mercaptoethanol (2-Me), 200-proof EtOH
- Dounce homogenizer
- ***User supplied reagents must be prepared fresh. This prevents absorption of ambient NH_3 which may introduce error in results.***

VI. Storage Conditions and Reagent Preparation:

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

- **CDA Assay Buffer:** Store at -20°C. Bring to RT before use. Precipitation is normal. Warm to 37°C and resuspend to dissolve.
- **CDA Substrate:** Resuspend in 140 µl dH_2O. Aliquot and store at -20°C. Freeze/thaw up to 3 times.
- **Cytidine Deaminase (CDA):** Reconstitute with 110 µl dH_2O. Aliquot and store at -80°C. Do not freeze-thaw multiple times.
- **Developer A:** Aliquot upon receiving the kit and store at -20°C. Keep on ice to prevent evaporation of the solvent.
- **Ammonium Chloride Standard (100 mM):** Aliquot and store at -20°C, bring to Room Temperature (RT) before use.
- **Plate sealer** (included).

VII. Cytidine Deaminase Activity Assay Protocol:

1. **Sample Preparation:** Add 100 µl of CDA Assay Buffer per 10 mg of sample (wet weight). Homogenize on ice using a Dounce homogenizer and stroke ~20 times. Let homogenized tissue sit on ice 10 minutes, then centrifuge at 10,000 x *g*, 4°C for 10 minutes. Collect the supernatant. *The sample may have a matrix effect on quantitativeness of the assay* (**Note a**). Add 2-30 µl sample per well and bring the volume to 40 µl with CDA Assay Buffer, and a parallel well with the same sample amount, and bring the volume of this well (sample background control well) to 50 µl with CDA Assay Buffer. For positive control: Dilute CDA 1:10: 5 µl of reconstituted CDA Stock + 45 µl of CDA Assay Buffer. Continue with further 8-fold dilution by adding 20 µl of 1:10 CDA to 140 µl of CDA Assay Buffer. Add 10 µl of diluted (1:80) CDA to a well, bring volume to 40 µl/well with CDA Assay Buffer.

   Notes:

a. 10K Spin Column: Dilute sample 1:4 with CDA Assay Buffer. Add to 10K Spin Column. Centrifuge the Spin Column at 10,000 x *g*, 4°C for 10 minutes. After each centrifugation, bring supernatant to original volume. Repeat dilution and centrifugation at least three times. After final centrifugation, bring retentate to original volume.

b. A pilot experiment is recommended to ensure sample RFU values are within the linear range of the standard curve. Add 2-30 µl of the serially diluted sample (1:10, 1:25, 1:50) to each well for evaluation.

c. Sample background control wells: **Do not add substrate to these samples**.

d. Use the Assay Genie BCA Protein Assay Kit II (BN01029) to determine the protein concentration of each homogenate.

2. **Ammonium Chloride Standard Curve:** Dilute Ammonium Chloride Standard Stock (100 mM) to 1 mM working solution (i.e. 5.0 µl 100 mM Stock + 495 µl of dH_2O). Then, dilute 1 mM working solution to 100 µM (i.e. 10 µl of 1 mM + 90 µl of dH_2O). Add 0, 2, 4, 6, 8, 10 µl of 100 µM Ammonium Chloride Standard to generate 0, 200, 400, 600, 800, 1000 pmoles of Ammonium Chloride Standard/well. Bring the volume of each well to 50 µl with CDA Assay Buffer. *Ammonia present in air can result in high background.*

3. **Diluted CDA Substrate:** Dilute CDA Substrate 20-fold to working concentration. Prepare 10 µl diluted CDA substrate/well (i.e. for 10 samples: 10 x 10 µl/well= 100 µl). To prepare 100 µl of diluted CDA Substrate at working concentration, add 5 µl of CDA Substrate + 95 µl dH_2O. Add 10 µl of substrate to each well and bring the volume to 50 µl with CDA Assay Buffer, see Step 1 of Protocol). **Seal plate with Microplate Sealing Film (provided)**. Incubate microplate for 30 minutes in a plate reader preheated to 37°C. Preset the plate reader to shake the plate every 5 minutes. While the plate is incubating, prepare the following two reagents as needed for the number of reactions to be evaluated:

   Developer B: Add 11 µl 2-Me to 1989 µl EtOH. Mix well. Keep on ice while in use.

   Reaction Mix: Prepare 150 µl Reaction Mix per well as follows:

	Reaction Mix
CDA Assay Buffer	136.0 µl
Developer A	7.0 µl
Developer B	7.0 µl

   Mix well. At the end of the 30 minute incubation time, add 150 µl of "Reaction Mix" to each well containing standards, samples, and sample background(s).

4. **Measurement:** Measure fluorescence at Ex/Em= 410/470 nm at 37°C in kinetic mode for 30 minutes.

   Note: We recommend measuring the RFU in kinetic mode, however the assay is an endpoint assay and the Ammonium Chloride Standard Curve should be applied to the RFU obtained at the end of the 30 minute time point.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Ammonium Chloride Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the Cytidine Deaminase activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the Ammonium Chloride Standard Curve to get B pmol of Ammonia generated during the reaction time ($\Delta T = t_2 - t_1$).

Sample Cytidine Deaminase Activity = B/(Δt X V) x D = pmol/min/ml = µU/ml

Where: **B** = Ammonia amount from Standard Curve (pmol).
Δt = reaction time (min.).
V = sample volume added into the reaction well (ml).
D = Dilution Factor

Unit Definition: One unit of Cytidine Deaminase is the amount of enzyme that converts 1.0 µmole of Ammonia per min. at pH 7.5 at 37°C.

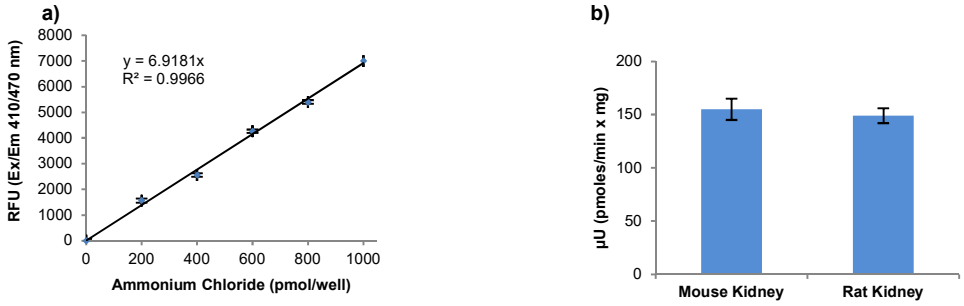

Figure: (**A**) Ammonium Chloride Standard Curve. (**B**) Estimation of Cytidine Deaminase Activity in mouse (26 µg) and rat (20 µg) kidney tissues. Mouse and rat kidney tissues were homogenized in CDA Assay Buffer with a dounce homogenizer. The supernatant was filtered three times with a 10 kD filter. Protein concentration was determined with BCA Assay Protein Quantitation Kit and CDA Activity determined following the kit protocol. For each sample, 2.5 µl of washed supernatant diluted 1:10 was analyzed. Data are an average of 4 replicates, each analyzed in two separate experiments.

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