



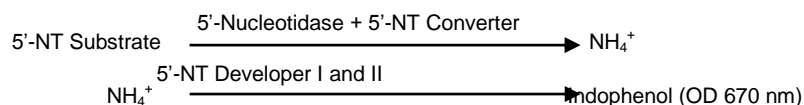
5'-Nucleotidase (CD73) Activity Assay Kit (Colorimetric)

7/17

(Catalog # K992-100; 100 assays; Store at -20°C)

I. Introduction:

5'-Nucleotidase (5'-NT), also known as CD73 (EC 3.1.3.5) is an enzyme located in the plasma membrane. It converts extracellular nucleotides like 5'-AMP to their corresponding nucleosides, through phosphorylitic cleavage. This conversion facilitates uptake of the nucleosides through nucleoside receptors into the cell, where they can again be phosphorylated to generate nucleotides and contribute to the nucleotide pool, inside the cell. 5'-NT levels are elevated in hepatic diseases such as viral hepatitis, alcoholic liver disease and cirrhosis. BioVision's 5'-Nucleotidase Activity Kit is a simple two-step end point assay that relies on the Berthelot's test for quantification of ammonia. In this assay, the action of 5'-nucleotidase on the substrate generates a product, which releases ammonia in presence of the converter. Developer I and II are then used to quantify the released ammonia through increase in absorbance at 670 nm. This assay can detect as low as 0.2 mU of 5'-NT. Since non-specific enzymes like alkaline phosphatase can give a positive signal in this assay, 5'-NT inhibitor may be used to completely inhibit 5'-nucleotidase and distinguish from the signal from non-specific enzymes. The assay kit also includes 5'-Nucleotidase (5'-NT) enzyme for use as positive control.



II. Applications:

- Measurement of 5'-Nucleotidase activity in various tissues/cells

III. Sample Type:

- Animal tissues lysate: eg. liver
- Cell lysate
- Recombinant enzyme, purified protein

IV. Kit Contents:

Components	K992-100	Cap Code	Part Number
5'-NT Assay Buffer	25 ml	WM	K992-100-1
5'-NT Substrate	1 vial	Blue	K992-100-2
5'-NT Converter	1 vial	Green	K992-100-3
5'-NT Inhibitor	250 µl	Orange	K992-100-4
5'-NT Stop Solution	500 µl	Red	K992-100-5
5'-NT Developer I	8 ml	Amber	K992-100-6
5'-NT Developer II	4 ml	Clear	K992-100-7
NH ₄ ⁺ Standard (100 mM)	100 µl	Yellow	K992-100-8
5'-NT Positive Control	1 vial	Purple	K992-100-9

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Incubator / water bath that can be heated to 37°C

VI. Storage Conditions and Reagent Preparation:

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

- **5'-NT Buffer:** Warm to room temperature before use.
- **5'-NT Substrate:** Reconstitute with 1.1 ml 5'-NT Assay Buffer. Aliquot and store at -20°C. Reconstituted substrate is stable for at least 2 months.
- **5'-NT Converter:** Stable for at least 3 months when stored at -20 °C. Reconstitute with 220 µl 5'-NT Assay Buffer before use. Gently pipette up and down to dissolve completely and then centrifuge briefly. Aliquot and store at -80°C. Use within two months. Keep on ice while in use.
- **5'-NT Positive Control:** Lyophilized enzyme is stable for at least 6 months when stored at -20 °C. Add 22 µl 5'-NT Buffer to the Positive Control and mix thoroughly. Aliquot and store at -80°C. Use within two months. Keep on ice while in use.
- All other components are ready to use after thawing.

VII. 5'-Nucleotidase Activity Assay Protocol:

1. Sample Preparation: Rapidly homogenize tissue (10 mg) or cells (1×10^6) with 100 µl ice cold 5'-NT Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube. Determine protein concentration using preferred method. *We recommend BV# K813-2500.* Protein concentration should range between 1-20 mg/ml. Concentrated samples may be diluted with 5'-NT assay buffer. Aliquot and store lysates at -80°C unless being used immediately. Use 5-20 µl sample per well. Prepare three identical wells for each sample labelled "Sample Background Control" (BC), "Sample" (S) and "Sample + Inhibitor" (SI). For SI well, add 5 µl 5'-NT Inhibitor in addition to sample. Adjust volume in each well to 50 µl with 5'-NT Assay Buffer.

