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Neuraminidase Activity Fluorometric Assay Kit

rev 12/19

(Catalog # K732-100; 100 assays; Store at -20°C)

I. Introduction:

Neuraminidase (NA) is a very common enzyme that hydrolyzes terminal sialic acid residues on polysaccharide chains, most often a galactose residue. NA activity plays a key role in the invasion of target cells and the replication of influenza virus. NA activity also assists in the elution of progeny viruses from infected cells and prevents the self-aggregation of virus. Thus, NA is an important target for drug development. **BioVision's Neuraminidase Activity Assay Kit** provides a simple and sensitive method for measuring NA activity using fluorescence (Ex/Em = 530/590 nm). The assay utilizes NA Probe to detect the neuraminidase activity. This high-throughput adaptable assay kit can detect NA activity as low as 2.0 mU/ml in a variety of samples.

II. Application:

- Measurement of NA activity in various tissues/cells extracts or serum sample infected with influenza virus.

III. Sample Type:

- Animal tissues: liver, brain, kidney etc.
- Cell culture: Adherent or suspension cells
- Serum

IV. Kit Contents:

Components	K732-100	Cap Code	Part Number
NA Assay Buffer	30 ml	NM	K732-100-1
NA Probe (in DMSO, anhydrous)	200 µl	Red	K732-100-2A
NA Substrate (Lyophilized)	1 vial	Blue	K732-100-3
NA Enzyme Mix I (Lyophilized)	1 vial	Green	K732-100-4
NA Enzyme Mix II (Lyophilized)	1 vial	Purple	K732-100-5
Galactose Standard (100 nmol/µl)	100 µl	Yellow	K732-100-6
NA Positive Control	100 µl	Orange	K732-100-7

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom, preferably black plate
- Multi-well spectrophotometer with fluorescence

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **NA Assay Buffer:** Warm Assay Buffer to room temperature (RT) before use. Store at -20°C. Use within two months.
- **NA Probe:** Warm to rRT before use. Store at -20°C. Use within two months.
- **NA Substrate:** Reconstitute with 110 µl dH_2O. Store at -20°C. Use within two months.
- **NA Enzyme Mix I:** Reconstitute with 220 µl NA Assay Buffer. Aliquot and store at -20°C. Stable for two months.
- **NA Enzyme Mix II:** Reconstitute with 220 µl NA Assay Buffer. Aliquot and store at -20°C. Stable for two months.
- **NA Positive Control:** Aliquot and store at -20°C. Use within two months.

VIII. Neuraminidase Assay Protocol:

1. **Sample Preparation:** Homogenize 10 mg of sample (wet weight or cell pellet) in 100 µl NA Assay Buffer. Centrifuge at 10,000 x g for 5 min. at 4°C. Collect the supernatant. Add 2-10 µl of supernatant or serum into a 96-well plate and adjust the volume to 50 µl with NA Assay Buffer. Add 10 µl of NA Positive Control into desired wells(s) and adjust the volume to 50 µl with NA Assay Buffer.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having background, prepare parallel well(s) containing the same amount of sample as in the test well (sample background control). Adjust the volume to 50 µl with NA Assay Buffer.

   c. 1% or higher Triton X-100 concentration interferes with the assay.

2. **Standard Curve Preparation:** Dilute Galactose Standard to 1 nmol/µl by adding 10 µl of 100 nmol/µl Galactose Standard into 990 µl of NA Assay Buffer, mix well. Dilute the Standard further to 0.1 nmol/µl by adding 20 µl of 1 nmol/µl Standard to 180 µl of NA Assay Buffer and mix. Add 0, 1, 2, 3, 4 and 5 µl of 0.1 nmol/µl Galactose Standard into a series of wells in 96-well plate* to generate 0, 0.1, 0.2, 0.3, 0.4 and 0.5 nmol/well of Galactose Standard. Adjust the volume to 50 µl/well with NA Assay Buffer.

   **** We recommend using black plate for the assay***

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples, Standards & Positive Control) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
NA Assay Buffer	44.5 µl	45.5 µl
NA Enzyme Mix I	2 µl	2 µl
NA Enzyme Mix II	2 µl	2 µl
NA Substrate	1 µl	----
NA Probe	0.5 µl	0.5 µl

Add 50 µl of Reaction Mix to each well containing the Standards, Positive Control and samples. Mix well. * For samples having background, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. **Measurement:** Incubate for 30 min at 37°C and measure fluorescence (Ex/Em = 535/590 nm) in kinetic mode.

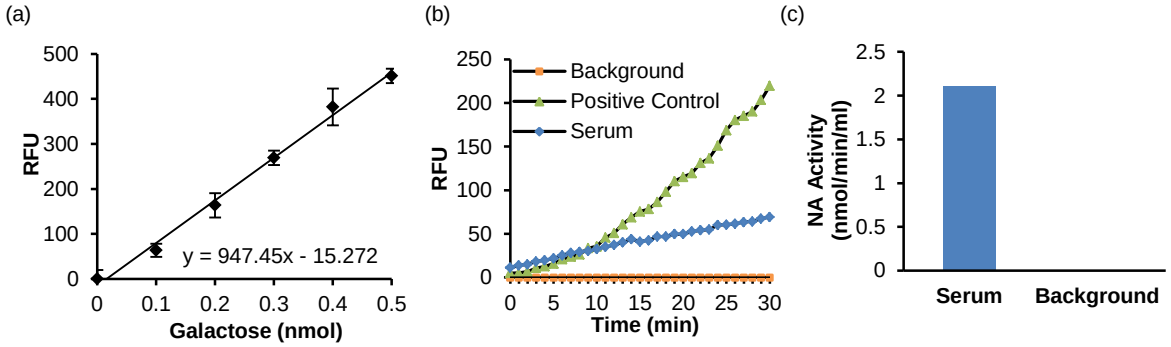
   Note: Incubation time depends on the NA Activity in the samples. Choose two time points (T_1 and T_2) in the linear range (fluorescence values A_1 and A_2 respectively) to calculate the NA activity of the samples. The Standard Curve can be read in end point mode (i.e. at the end of incubation time).

5. **Calculations:** Subtract 0 Standard reading from all readings. Plot the Galactose Standard Curve. If sample background control reading is significant, subtract background control reading from sample readings. Calculate the NA activity of the test sample: $\Delta RFU = A_2 - A_1$. Apply ΔRFU to the Standard Curve to get B nmol of Galactose generated by NA during the reaction time ($\Delta T = T_2 - T_1$).

   Sample Neuraminidase Activity = B/(ΔT X V) x D = nmol/min/ml = mU/ml

   Where: **B** is the Galactose amount from the Standard Curve (nmol)
   ΔT is the reaction time (min.)
   V is the sample volume added into the reaction well (ml)
   D is the sample dilution factor

   Unit Definition: One unit of Neuraminidase activity is the amount of enzyme that generates 1.0 µmol of Galactose per min. at pH 7.4 at 37°C.



Figures: (a) Galactose Standard Curve. (b) NA activity in normal human serum (1 µl) & Positive Control (1 µl). (c) Calculated activity of serum. Assays were performed following the kit protocol.

IX. Related Products:

Amylase Activity Colorimetric Assay Kit (K711)
α-Glucosidase Activity Colorimetric Assay Kit (K690)

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