Neuraminidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1333

(Version 1.1A)

Detection and Quantification of Neuraminidase (NA) Activity in Cell lysate, Viral culture supernatant and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Neuraminidase (NA, also known as sialidase) is a critical surface glycoprotein of enveloped viruses, most notably influenza viruses, which hydrolyzes sialic acid residues from host cells and viral glycoproteins. During the viral replication cycle, NA cleaves sialic acid receptors on the cell surface to prevent self-aggregation of nascent virions, thereby facilitating efficient viral release and spread. As one of the two major antigens of influenza viruses, NA enzymatic activity is essential for viral pathogenicity and transmissibility, and serves as the primary target for antiviral drugs such as oseltamivir.

Neuraminidase Activity Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of neuraminidase activity in a variety of samples. In this assay, neuraminidase hydrolyzes terminal sialic acid residues on poly-saccharide chains. H_2O_2 is generated through a linked enzymatic reaction, which reacts with dye reagent to a colored product. The color intensity increase at 570nm is proportional to the Neuraminidase activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer A	9 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Reaction Buffer B	10 ml x 1	4 °C
Diluent	5 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	1 ml x 1	4 °C
Standard	0.2 ml x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note: Avoid freeze-thaw cycles

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Transfer 20 µl Standard into 960 µl distilled water and mix well to generate 200 mmol/L as the stock standard solution, which could be stored at 4°C for 2 weeks. Then add 2 µl stock solution into 998 µl distilled water to generate 400 µmol/L as the top standard solution. Then perform 2-fold serial dilutions of the top solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 µmol/L.

Substrate: Briefly centrifuge prior to opening. Dissolve in 2 ml distilled water before use. Store at 4 °C for 5-6 days or -20°C for 3-4 month after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Dissolve in 2 ml Diluent before use. Keep in dark and store at 4 °C for 1-2 weeks or -20°C for 1-2 months after reconstitution.

Dye Reagent Working Solution: Warm Dye Reagent Diluent to RT before use. Briefly centrifuge the vial of Dye Reagent prior to opening. Dissolve in 0.2 ml Dye Reagent Diluent before use to generate Dye Reagent stock solution. Keep in dark and store at -20 °C for 4-5 months or 4°C for 3 days. Dilute the stock solution 10-fold using Diluent to prepare the Dye Reagent Working Solution (eg. 10 µl into 90 µl Diluent). Prepare working solution fresh for immediate use.

Positive Control: Briefly centrifuge prior to opening. Dissolve in 1 ml Reaction Buffer A before use. Store at 4 °C for 1 week or -20 °C for 3-4 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For Cell lysate samples

Detect directly or dilute with Assay Buffer.

2. For Viral culture supernatant samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Positive Control	Control	Standard	Blank
Reaction Buffer A	70 µl	70 µl	70 µl	--	--
Substrate	20 µl	20 µl	20 µl	--	--
Sample	10 µl	--	--	--	--
Positive Control	--	10 µl	--	--	--
Distilled water	--	--	10 µl	110 µl	120 µl
Mix and put in the oven at 37 °C for 30 minutes					
Reaction Buffer B	70 µl	70 µl	70 µl	70 µl	70 µl
Enzyme	20 µl	20 µl	20 µl	--	--
Standard	--	--	--	10 µl	--
Dye Reagent Working Solution	10 µl	10 µl	10 µl	10 µl	10 µl
Mix and put in the oven at 37 °C for 5 minutes, record absorbance measured at 570 nm					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of Neuraminidase is defined as the enzyme generates 1 μmol H_2O_2 per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times n \quad (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \quad (U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

V_{Sample}: the volume of sample in assay procedure, mL

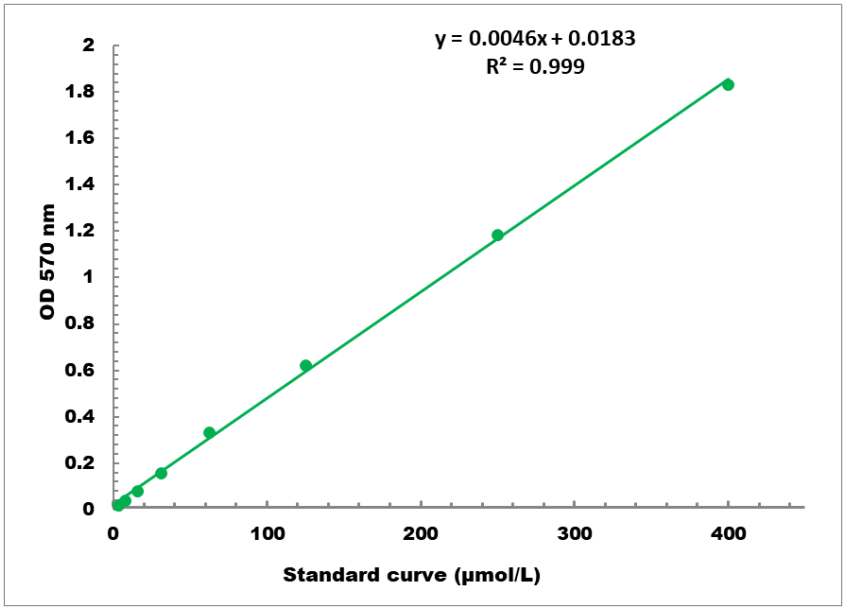
V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, 30 minutes

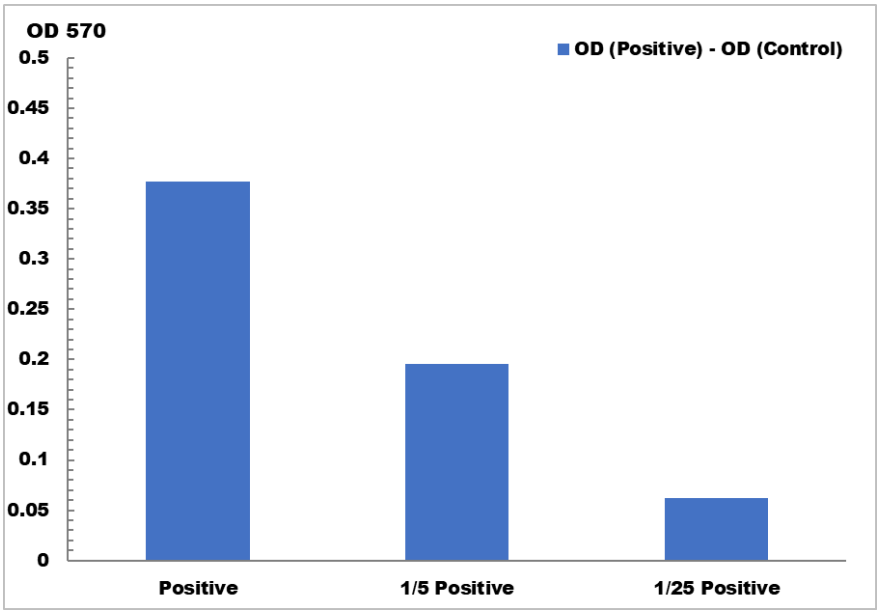
V: the volume of sample in sample preparation, ml

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration