# ab185436

# Neuraminidase Activity Assay Kit (Fluorometric)

## Instructions for Use

For the rapid, sensitive and accurate measurement of Neuraminidase activity in various samples.

This product is for research use only and is not intended for diagnostic use.

Version: 1 Last Updated: 18 March 2014

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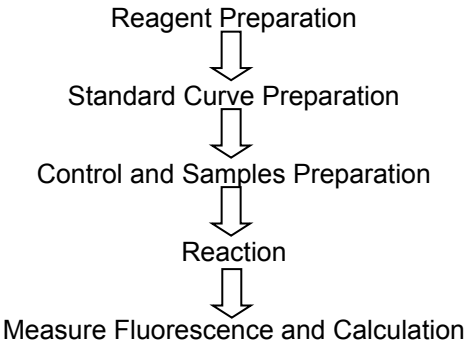
# 1. Overview

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 self-aggregation of virus. Thus, NA is an important target for drug development.

Abcam's Neuraminidase Activity Assay Kit (Fluorometric) (ab185436) 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.

## 2. Protocol Summary



# 3. Kits Components

| Item | Quantity |
|---|---|
| NA Assay Buffer | 30 mL |
| NA Probe | 200 µL |
| NA Enzyme Mix I | 1 vial |
| NA Enzyme Mix II | 1 vial |
| Galactose Standard (100 nmol/µL) | 100 µL |
| NA Positive Control | 100 µL |
| NA Substrate | 1 vial |

## 4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

## 5. Materials Required, Not Supplied

- Distilled water ($dH_2O$)
- 96-well black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

# 6. Reagents Preparation

1. **NA Assay Buffer and NA Probe:**
   Warm to room temperature before use. Aliquot and store at -20°C. Stable for two months.

2. **NA Substrate:**
   Reconstitute with 110 µL $dH_2O$. Aliquot and store at -20°C. Stable for two months.

3. **NA Positive Control:**
   Aliquot and store at -20°C. Use within two months

4. **NA Enzyme Mix I and Enzyme Mix II:**
   Reconstitute with 220 µL NA Assay Buffer. Aliquot and store at -20°C. Stable for two months.

# 7. Assay Protocol

**1. Sample Preparation**

a) Homogenize 10 mg of sample (wet weight or cell pellet) in 100 µL NA Assay Buffer.

b) Centrifuge at 10,000 x *g* for 5 minutes at 4°C. Collect the supernatant.

c) Add 2-10 µL of supernatant or serum into a 96-well plate and adjust the volume to 50 µL with NA Assay Buffer.

d) Add 10 µL of NA Positive Control into desired wells(s) and adjust the volume to 50 µL with NA Assay Buffer.

*NOTE:*

- *For unknown samples, we suggest performing pilot experiment & testing several doses of your samples to ensure the readings are within the Standard Curve range.*
- *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.*
- *1% or higher Triton X-100 concentration interferes with the assay.*

**2. Standard Curve Preparation:**

**a)** 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.

**b)** 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.

**c)** Adjust the volume to 50 µL/well with NA Assay Buffer.

**3. Reaction Mix:**

Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 50 µL 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 minutes at 37°C and measure fluorescence (Ex/Em = 535/590 nm) kinetically.

*NOTE:*

- *Incubation time depends on the NA Activity in the samples. Choose two time points (T1 and T2) in the linear range (fluorescence values A1 and A2 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).*

# 8. Data Analysis

**Calculations:**

a) 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: ΔRFU = A2 – A1. Apply ΔRFU to the Standard Curve to get B nmol of Galactose generated by NA during the reaction time (ΔT = T2 – T1).

**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 (minutes)

**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.

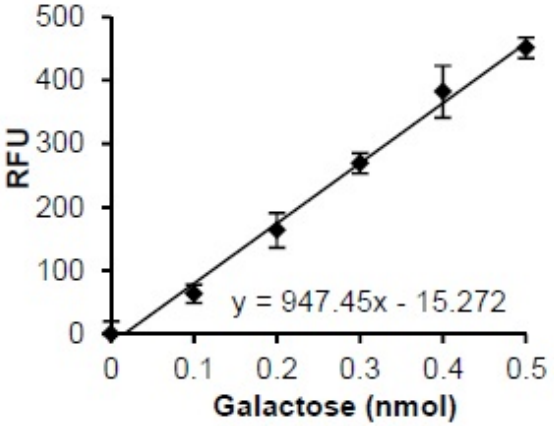



**Figure 1.** Galactose Standard Curve.

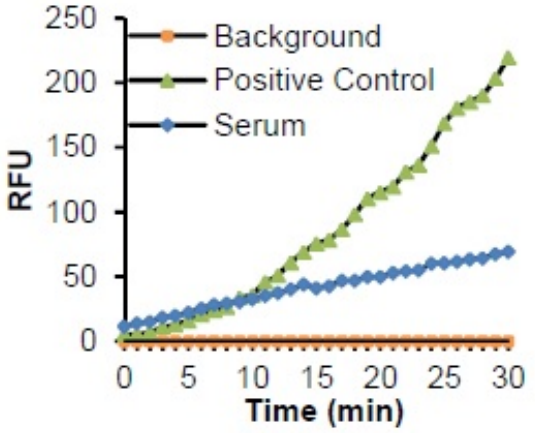



**Figure 2.** NA activity in normal Human serum (1 µL) and positive control (1 µL). Assays were performed following the kit protocol.

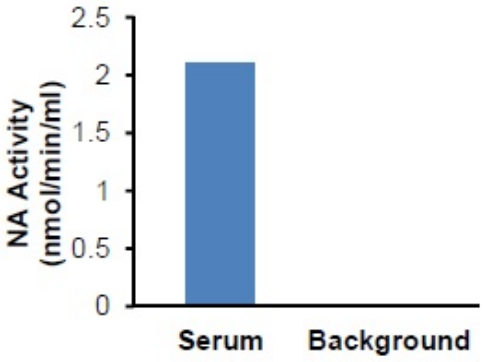



**Figure 3.** Calculated activity of serum. Assays were performed following the kit protocol.

# 9. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| Assay not working | Assay buffer at wrong temperature | Assay buffer must not be chilled - needs to be at RT |
| | Protocol step missed | Re-read and follow the protocol exactly |
| | Plate read at incorrect wavelength | Ensure you are using appropriate reader and filter settings (refer to datasheet) |
| | Unsuitable microtiter plate for assay | Fluorescence: Black plates (clear bottoms);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>If critical, datasheet will indicate whether to use flat- or U-shaped wells |
| Unexpected results | Measured at wrong wavelength | Use appropriate reader and filter settings described in datasheet |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Unsuitable sample type | Use recommended samples types as listed on the datasheet |
| | Sample readings are outside linear range | Concentrate/ dilute samples to be in linear range |

| Problem | Reason | Solution |
|---|---|---|
| Samples with inconsistent readings | Unsuitable sample type | Refer to datasheet for details about incompatible samples |
| | Samples prepared in the wrong buffer | Use the assay buffer provided (or refer to datasheet for instructions) |
| | Samples not deproteinized (if indicated on datasheet) | Use the ***10kDa spin column (ab93349)*** |
| | Cell/ tissue samples not sufficiently homogenized | Increase sonication time/ number of strokes with the Dounce homogenizer |
| | Too many freeze-thaw cycles | Aliquot samples to reduce the number of freeze-thaw cycles |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Samples are too old or incorrectly stored | Use freshly made samples and store at recommended temperature until use |
| Lower/ Higher readings in samples and standards | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Out-of-date kit or incorrectly stored reagents | Always check expiry date and store kit components as recommended on the datasheet |
| | Reagents sitting for extended periods on ice | Try to prepare a fresh reaction mix prior to each use |
| | Incorrect incubation time/ temperature | Refer to datasheet for recommended incubation time and/ or temperature |
| | Incorrect amounts used | Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume) |

| Problem | Reason | Solution |
|---|---|---|
| Standard curve is not linear | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Pipetting errors when setting up the standard curve | Try not to pipette too small volumes |
| | Incorrect pipetting when preparing the reaction mix | Always prepare a master mix |
| | Air bubbles in wells | Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates |
| | Concentration of standard stock incorrect | Recheck datasheet for recommended concentrations of standard stocks |
| | Errors in standard curve calculations | Refer to datasheet and re-check the calculations |
| | Use of other reagents than those provided with the kit | Use fresh components from the same kit |

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