Description
The NEU2 Fluorogenic Assay Kit is designed to measure NEU2 (Neuroaminidase 2 or Sialidase-2) glycohydrolase activity for screening and profiling applications. It comes in a convenient 96-well format, with purified recombinant NEU2 enzyme, NEU2 substrate, NEU2 assay buffer, and Stop Solution for 100 enzyme reactions. The NEU2 inhibitor NCGC00063279 is also included as a control.

Hydrolysation of the NEU substrate releases a fluorophore (λexc/λem = 365 nm/445 nm). The increase in fluorescence at λ=445 nm is directly proportional to NEU2 activity.

Background
NEU2, also known as N-acetyl-alpha-neuroaminidase 2 or sialidase-2, belongs to the glycohydrolytic enzyme family. Glycoside hydrolases remove terminal sialic acid residues from sialo-glycoproteins and sialo-glycolipids. The human cytosolic sialidase NEU2 recognizes not only the a(2-3), a(2-8), and a(2-6) of the sialosyl linkage of the terminal sialic acid residue but also the inner sugar(s) of the oligosaccharide moiety, as well as the supramolecular organization of gangliosides. NEU2 has been implicated in several diseases such as cancer and type I and type II sialidosis. The PI3K-AKT pathway is upregulated in many cancers, with many of the proteins involved in this pathway being altered by sialylation, which can result in oncogene inactivation. Lower than average levels of NEU2 have been found in pancreatic cancer cells, resulting in decreased apoptosis. Inhibition of NEU2 is an important approach to decipher sialidase function and mechanism of action. The use of inhibitors may also prove useful as a therapeutic approach.

Applications
Enzyme kinetics studies and screening small molecule inhibitors for drug discovery and high-throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
101722	NEU2, His-Tag*	3 µg	-80°C
	NEU2 Assay Buffer	5 ml	-20°C
	25 mM NEU2 Substrate	10 µl	-20°C
	5x Stop Solution	3 ml	-20°C
	N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid	300 µg	-20°C
79685	96-well black microplate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied
Fluorescent microtiter plate reader capable of measurement at λex355-375/λem435-455 nm.
Orbital shaker.

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

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Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Negative Control”, “Positive Control”, “Control Inhibitor”, and “Test Inhibitor” conditions.
- If the assay plate is going to be used more than once, prepare enough of each reagent for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or as recommended for each reagent.
- ***Unused diluted proteins should be discarded.***

1. Dilute **25 mM NEU2 Substrate** 50-fold with **NEU2 Assay Buffer** to make a 500 µM solution.

2. Add 5 µl of diluted **NEU2 Substrate** to the “Positive Control”, “Test Inhibitor”, “Control Inhibitor”, and “Negative Control” wells.

3. Thaw **NEU2** on ice. Briefly spin the tube to recover its full content.

4. Dilute NEU2 to 2 ng/µl with NEU2 Assay Buffer (15 µl/well).

   Note: Keep the diluted protein on ice until use. Do not freeze and re-use the diluted protein.

5. Add 15 µl of diluted enzyme to the “Positive Control”, “Control Inhibitor” and “Test Inhibitor” wells.

6. Resuspend 300 µg of N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid with 20.6 ml of NEU2 Assay Buffer to make a 50 mM solution.

7. Add 5 µl to each well designated “Control Inhibitor”.

8. Prepare the Test inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   8.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in NEU2 Assay Buffer at concentrations 5-fold higher than the desired final concentrations.

   OR

   8.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 20-fold in NEU2 Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 5%.

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Using NEU2 Assay Buffer containing 5% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations.

For positive and negative controls, prepare 5% DMSO in NEU2 Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO in the assay should not exceed 1%.

9. Add 5 µl of Test inhibitor to each well designated “Test Inhibitor”.

10. Add 20 µl of NEU2 Assay Buffer to the “Blank” wells.

11. Add 15 µl of NEU2 Assay Buffer to the “Negative Control”.

12. Add 5 µl of Diluent Solution to the “Positive Control”, “Negative Control”, and “Blank” wells.

13. Incubate at Room Temperature (RT) for 20 minutes with gentle agitation.

14. Prepare 1x Stop Solution by adding 1 part of 5x Stop Solution and 4 parts of distilled water. Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 5x Stop Solution and store at -20°C.

15. After 20 minutes, stop the reaction by adding 125 µl of 1x Stop Solution.

16. Incubate for 5-10 minutes at RT with gentle agitation.

17. Read the fluorescence intensity of the samples (λexcitation = 355-375 nm; λemission = 435-455 nm) in a fluorescence reader.

18. The “Blank” value should be subtracted from all other values.

Component	**Blank**	**Negative control**	**Positive Control**	**Control Inhibitor**	**Test Inhibitor**
Diluted NEU2 Substrate (500 µM)	-	5 µl	5 µl	5 µl	5 µl
Diluted NEU2 (2 ng/µl)	-	-	15 µl	15 µl	15 µl
N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (50 mM)	-	-	-	5 µl	-
Test Inhibitor	-	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-	-
NEU2 Assay Buffer	20 µl	15 µl	-	-	-
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**	**25 µl**

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Example Results

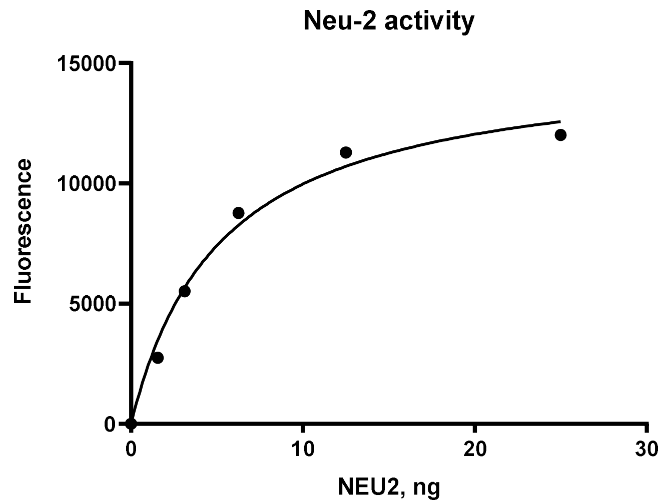

Figure 1. NEU2 activity.
NEU2 activity was measured in the presence of increasing amounts of NEU2.

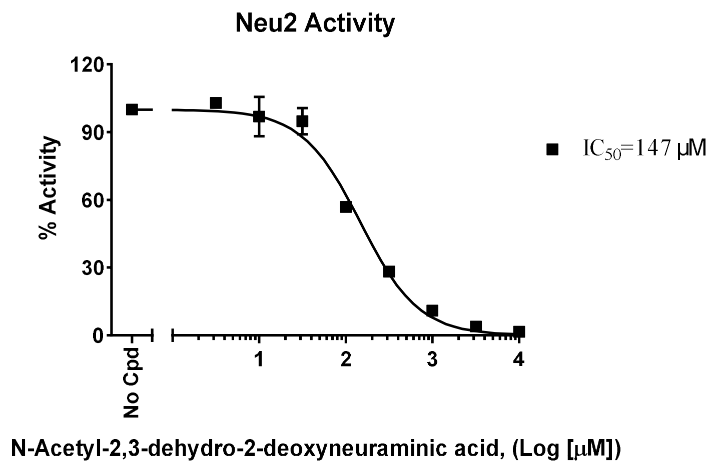

Figure 2. Inhibition of NEU2 activity by N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid.
NEU2 activity was measured in the presence of increasing concentrations of N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (Sigma #D9050). Results are expressed as percent activity, in which the activity of NEU2 in the absence of inhibitor is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Khedri Z, *et al.*, 2012, *Org. Biomol. Chem* 10: 6112-6120.
Mozzi A, *et al.*, 2011, *Proteins* 80: 1123-1132.
Nath S., *et al.*, 2018, *Cell Death and Disease* 9: 210.

Related Products

Products	*Catalog #*	*Size*
Sialidase, His-Tag Recombinant	101734	25 µg/100 µg
Caspase3, His-Tag Recombinant	80500	10 µg/50 µg
Caspase6, His-Tag Recombinant	80113	10 µg/50 µg
Caspase8, His-Tag Recombinant	80114	10 µg/50 µg

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