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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Sialidase Fluorogenic Assay Kit is designed to measure Sialidase NANH (N-acylneuraminate glycohydrolase) activity for screening and profiling applications. It comes in a convenient 96-well format, with purified recombinant *Salmonella typhimurium* sialidase, Sialidase substrate, Sialidase assay buffer, and Stop Solution for 100 enzyme reactions. Sialidase inhibitor NCGC00063279 (N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid) is also included as a control.

The Sialidase fluorogenic substrate is incubated with Sialidase. Hydrolyzation of the Sialidase substrate releases a fluorophore (λexc/λem = 365 nm/445 nm). The increase in fluorescence at λ=445 nm is directly proportional to Sialidase activity.

Background

Sialidases are glycosyl hydrolases that release α-2, 3-, α-2, 6- and α-2, 8-glycosidic linkages of terminal sialic acid residues from oligosaccharides, glycoproteins, and glycolipids. The *Salmonella typhimurium* sialidase NANH has been shown to have a high K_M value (mM range) against polyvalent targets and plays a role in enhancement of NK cell-mediated antibody-dependent cellular cytotoxicity. *Salmonella typhimurium* sialidase is thought to be an immune-modulatory enzyme, therefore, its regulation may be important for therapy of diseases associated with disorders of the immune system.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
101734	Sialidase, His-Tag*	2 µg	-80°C
	Sialidase Assay Buffer	5 ml	-20°C
	25 mM Sialidase Substrate	10 µl	-20°C
	5x Stop Solution	3 ml	-20°C
	NCGC00063279	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 platform.

Stability

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

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.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

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

2. Add 5 µl of diluted Sialidase Substrate to "Negative Control", "Positive Control", "Control Inhibitor", and "Test Inhibitor".

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

4. Dilute **Sialidase** to 0.05 ng/µl with Sialidase Assay Buffer (15 µl/well).

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

6. Add 15 µl of Sialidase Assay Buffer to the "Negative Control" wells.

7. Add 20 µl of Sialidase Assay Buffer to the "Blank" wells.

8. Resuspend 300 µg of NCGC00063279 with 20.6 µl of distilled water to make a 50 mM solution.

9. Add 5 µl to each well designated "Control Inhibitor".

10. 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.

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

    For the positive and negative controls, use 1x Cathepsin Buffer (Diluent Solution).

    OR

    10.2 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 Sialidase Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 5%.

Using Sialidase 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 Sialidase 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%.

11. Add 5 µl of Test inhibitor to each well designated "Test Inhibitor".
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.
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 Sialidase Substrate (500 µM)	-	5 µl	5 µl	5 µl	5 µl
Diluted Sialidase (0.05 ng/µl)	-	-	15 µl	15 µl	15 µl
NCGC00063279 (50 mM)	-	-	-	5 µl	-
Test Inhibitor	-	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-	-
Sialidase Assay Buffer	20 µl	15 µl	-	-	-
Reaction	**25 µl**	**25 µl**	**25 µl**	**25 µl**	**25 µl**

Example Results

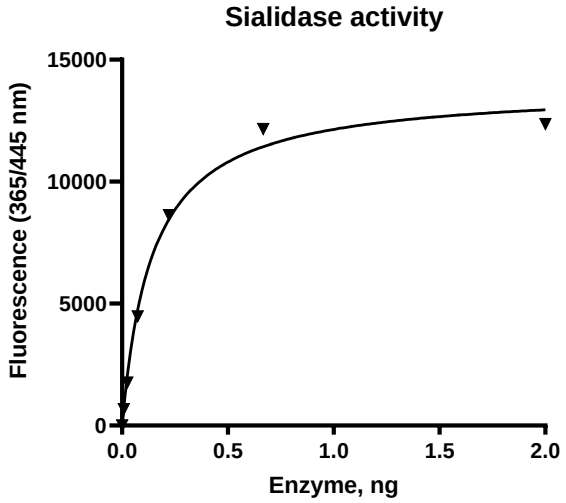

Figure 1. Sialidase activity.
Sialidase activity was measured in the presence of increasing Sialidase.

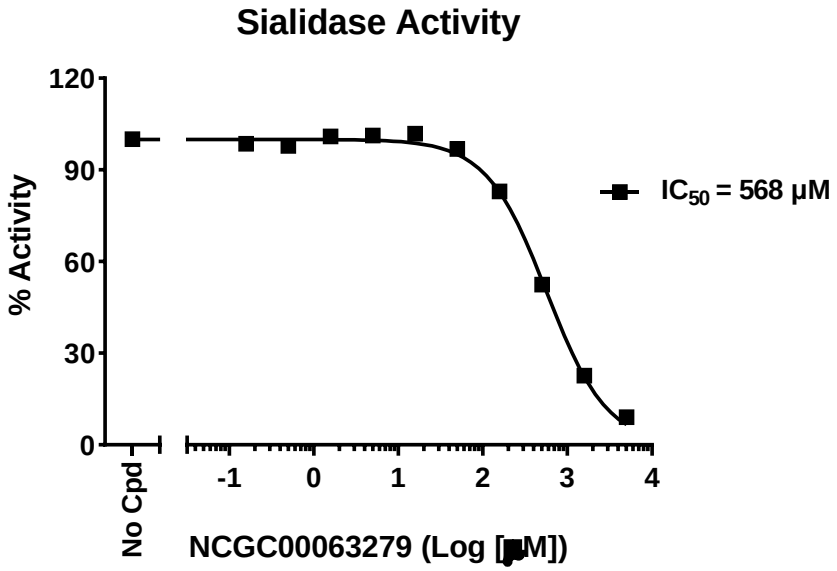

*Figure 2. Inhibition of S*ialidase *activity by NCGC00063279.*
Sialidase activity was measured in the presence of increasing concentrations of inhibitor. Results are expressed as percent activity, in which the activity of Sialidase in the absence of inhibitor is set to 100%.

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

Troubleshooting Guide

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

References

Gray MA, *et al.,* 2020, *Nat. Chem. Biol.* 16: 1376-1384
Szijj PA, *et al.*, 2023, *Nat. Chem.* doi: 10.1038/s41557-023-01280-4.

Related Products

Products	*Catalog #*	*Size*
NEU2 Fluorogenic Assay Kit	82139	96 reactions
NEU2, His-Tag Recombinant	101722	25 µg