ab83375 - Sialic Acid (NANA) Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For rapid, sensitive and accurate measurement of Sialic Acid (NANA) in various samples.

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

Version 7a Last Updated 30 June 2025

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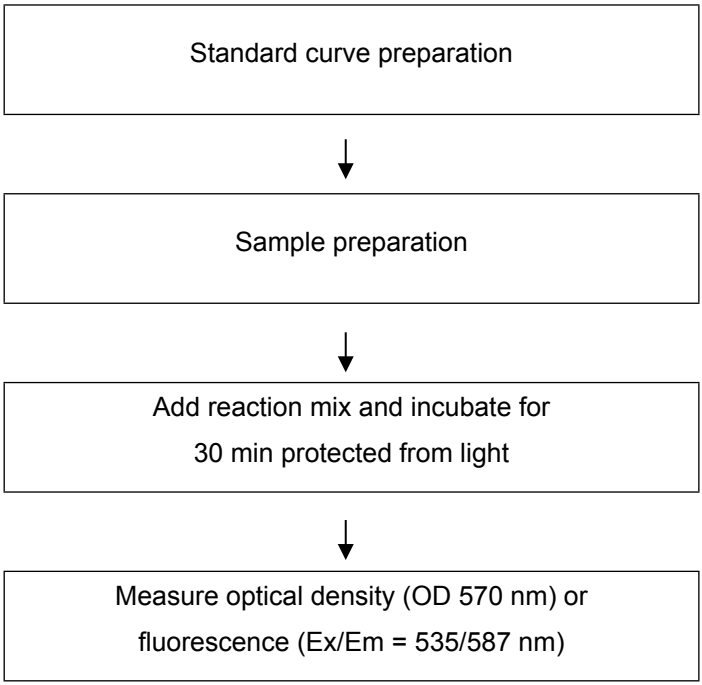
1. BACKGROUND

Sialic Acid Assay Kit (ab83375) is a simple and convenient kit for measuring free Sialic Acid (mainly N-acetylneuraminic acid or NANA) in a variety of biological samples. The detection is based in an enzyme coupled reaction in which oxidation of free sialic acid creates an intermediate that reacts stoichiometrically with the probe to generate a product that can detected by absorbance (OD = 570 nm) or fluorescence (Ex/Em=535/587 nm).

The kit measures sialic acid in the linear range of 0.1 to 10 nmol with a detection sensitivity ~1 µM concentration.

Sialic acid is a generic term for the N- or O-substituted derivatives of neuraminic acid, a monosaccharide with a nine-carbon backbone. It is also the name for the most common member of this group, N-acetylneuraminic acid (NANA). Sialic acids are found widely distributed in animal tissues and to a lesser extent in other species ranging from plants and fungi to yeasts and bacteria, mostly in glycoproteins and gangliosides. It has been shown recently that sialic acid level may be associated with developmental and pathological stages.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 4	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Sialic Acid Converting Enzyme	1 vial	-20°C	-20°C
Developer Mix A	1 vial	-20°C	-20°C
Sialic Acid Standard	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and Sialic Acid Assay Buffer, and Developer Mix A as Development Enzyme Mix I and Sialic Acid Development Mix.
OxiRed™ Probe was previously labelled as OxiRed Probe and Sialic Acid Probe (in DMSO).
The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Heat block or water bath

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 4:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **OxiRed™ Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot OxiRed™ Probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Once the OxiRed™ Probe is thawed, use with two months

9.3 **Sialic Acid Converting Enzyme:**

Reconstitute enzyme with 220 µL Assay Buffer 4. Pipette up and down to dissolve. Aliquot converting enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Once thawed, use within two months. Avoid repeated freeze/thaw cycles. Keep on ice during use.

9.4 **Developer Mix A:**

Reconstitute with 220 µL Assay Buffer 4. Pipette up and down to dissolve. Aliquot Developer Mix A so that you have enough volume to perform the desired number of assays. Store at -20°C. Once thawed, use within two months. Avoid repeated freeze/thaw cycles. Keep on ice during use.

9.5 **Sialic Acid Standard:**

Reconstitute the Sialic Acid Standard provided with 100 µL of ddH_2O to generate a 100 mM standard stock solution (100nmol/µL). Pipette up and down to dissolve. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Once thawed,

use within two months. Avoid repeated freeze/thaw cycles. Keep on ice during use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable cannot be stored for future use.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 500 µL of 1mM standard Sialic Acid, by diluting 5 µL of the 100 mM standard with 495 µL ddH_2O.

10.1.2 Using 1mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 4 (µL)	Final volume standard added to each well (µL)	End [Sialic Acid] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 **For the flurometric assay:**

10.2.1 Prepare 500 µL of 1mM standard Sialic Acid as described in Section 10.1.1.

10.2.2 Prepare 500 µL of 0.1 mM standard by diluting 50 µL of 1mM standard in 450 µL ddH_2O.

10.2.3 Using 0.1 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 4 (µL)	Final volume standard added to each well (µL)	End Sialic Acid con in well
1	0	150	50	0 nmol/well
2	6	144	50	0.2 nmol/well
3	12	138	50	0.4 nmol/well
4	18	132	50	0.6 nmol/well
5	24	126	50	0.8 nmol/well
6	30	120	50	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. Alternatively, if that is not possible, we suggest that you snap freeze and store the samples immediately after extraction at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 **Cell or tissue culture supernatant or media samples:**

Collect the sample necessary for each assay and use directly.

If you want to measure bound sialic acid found in your sample, please follow procedure described in section 11.3.

11.2 **Urine and other biological fluids:**

There is not much free sialic acid in plasma, therefore it is unlikely this product will work in plasma or serum.

Urine is a better sample and it can be used directly in the assay.

If you want to measure bound sialic acid found in your sample, please follow procedure described in section 11.3.

11.3 **Bound Sialic Acid hydrolysis step:**

11.3.1 Starting material can be glycoprotein, glycolipid or glycan.

11.3.2 Dissolve sample in a final concentration of 2 M acetic acid and heat to 80°C for 3 hours to release sialic acids.

11.3.3 Add 2 M NaOH to neutralize the solution.

11.3.4 Collect released sialic acids by ultra-centrifugation through a 3,000 MWCO filter.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 Set up Reaction wells:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 - 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 4).
- Background control sample wells= 1 - 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 4). ***NOTE****: for samples with high pyruvate content as it will generate background*.

12.2 Reaction Mix (COLORIMETRIC ASSAY):

Dilute the samples in Assay Buffer 4 1:1 then prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 4	**44**	**46**
Sialic Acid Converting Enzyme	**2**	**0**
Developer Mix A	**2**	**2**
OxiRed™ Probe	**2**	**2**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:
X µL component x (Number samples + standards +1)

12.3 **Reaction Mix (FLUOROMETRIC ASSAY):**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 4	**45.8**	**47.8**
Sialic Acid Converting Enzyme	**2**	**0**
Developer Mix A	**2**	**2**
OxiRed™ Probe*	**0.2**	**0.2**

**For fluorometric readings, using 0.2 µL/well of the OxiRed™ Probe decreases the background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.4 Add 50 µL of the appropriate reaction mix to each well containing standard and samples. Mix well.

12.5 Incubate at room temperature for 30 minutes protected from light.

12.6 Measure output on a microplate reader.

- Colorimetric assay: measure OD 570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of Sialic Acid.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Extrapolate sample readings from the standard curve plotted using the following equation:

$$A = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.7 Concentration of Sialic Acid (nmol/µL or mM) in the test samples is calculated as:

$$Sialic\ acid\ concentration = \left(\frac{A}{B}\right) * D$$

Where:

A = Amount of Sialic acid in the sample well (nmol).

B = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

Sialic acid molecular weight is 309.3 g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

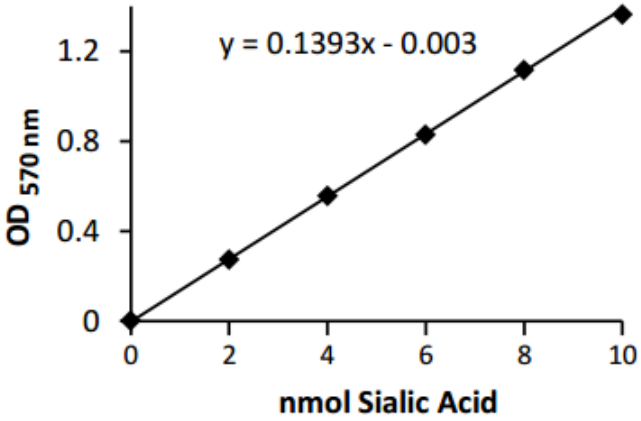


Figure 1. Typical sialic acid standard calibration curve using colorimetric reading.

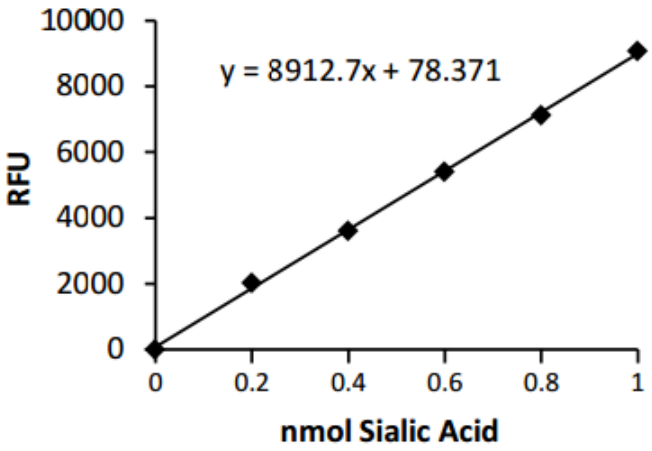


Figure 2. Typical sialic acid standard calibration curve using fluorometric reading.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, OxiRed™ Probe, converting enzyme and Developer Mix A; get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Sialic Reaction Mix (Number samples + standards + 1).

Component	Colorimetric / Backg Reaction Mix (µL)	Fluorometric / Backg Reaction Mix (µL)
Assay Buffer 4	**44 / 46**	**45.8 / 47. 8**
Sialic Acid Converting Enzyme	**2 / 0**	**2 / 0**
Developer Mix A	**2 / 2**	**2 / 2**
OxiRed™ Probe	**2 / 2**	**0.2 / 0.2**

- Add 50 µL of Reaction Mix to the standard and sample wells.
- Incubate plate at RT 30 mins protected from light.
- Measure plate at OD 570 nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.FAQ

Can I use this product to measure sialic acid in cells or tissue?

This product hasn't been tested on cell lysates. In theory, you could use the Assay Buffer to lyse the cells. However, you will need to choose a specific neuraminidase in order to cleave bound sialic acid off the glycoproteins and other complex carbohydrates to be able to use this kit, as most sialic acid found in cells in bound and not free.

You will need to choose the specific neuraminidase yourself because different neuraminidases will cleave at different sites depending on the targets.

18.NOTE

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)