EnzyChrom™ Sialic Acid Assay Kit (Cat# ESLA-100)

Quantitative Colorimetric/Fluorimetric Sialic Acid Determination

DESCRIPTION

SIALIC ACID is a general name for nine carbon acidic sugars with N- or O-substituted derivatives. The most common member of these sugars is N-acetylneuraminic acid (NANA). Sialic acid is widely distributed throughout mammalian tissues and fluids including serum. Sialylated oligosaccharides have been shown to exhibit antiviral properties and are also known to influence blood coagulation and cholesterol levels. The Sialic acid level in body fluids is also an important marker for diagnosing cancer. Simple, direct and automation-ready procedures for measuring sialic acid concentrations find wide applications in research and drug discovery. BioAssay Systems' sialic acid assay uses a single Working Reagent that combines NANA aldolase, pyruvate oxidase and hydrogen peroxide determination in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λem/ex = 585/530nm is directly proportional to sialic acid concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 0.02 to 1 mM sialic acid for colorimetric assays and 2 to 100 µM for fluorimetric assays.

Simple and convenient. Can detect free sialic acid by addition of a single working reagent and incubation for 60 min at room temperature or total sialic acid by pre-treating samples with a 60 min hydrolysis step.

APPLICATIONS

Direct Assays: sialic acid in biological samples.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer:	10 mL	**Hydrolysis Reagent:**	10 mL
Enzyme:	120 µL	**Neutraliztion Reagent:**	5 mL
Dye Reagent:	120 µL	**Standard:**	500 µL 10 mM Sialic Acid

Storage conditions. The kit is shipped on ice. Store all kit components at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

BOUND SIALIC ACID HYDROLYSIS PROCEDURE

Note: For measurement of free sialic acid, this procedure should be skipped.

1. Combine 20 µL of sample with 80 µL Hydrolysis Reagent in a microcentrifuge tube (screw cap tube is preferable) and incubate at 80°C for 60 min.
2. Allow sample to cool to room temperature and briefly centrifuge at 14000 rpm to spin down the hydrolysis mixture.
3. Add 20 µL Neutralization Reagent to each hydrolysis reaction, briefly vortex to mix and briefly centrifuge at 14000 rpm to spin down the reaction. The samples are now ready for the sialic acid assay.

COLORIEMTRIC PROCEDURE

Note: SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to room temperature. Prepare a 1 mM Standard Premix by mixing 50 µL of the 10 mM Standard and 450 µL dH_2O. Dilute Standard in distilled water as follows.

No	Premix + H_2O	Vol (µL)	Sialic Acid (mM)
1	100µL + 0µL	100	1.0
2	60µL + 40µL	100	0.6
3	30µL + 70µL	100	0.3
4	0µL + 100µL	100	0

Transfer 10 µL standards and 10 µL samples into separate wells of a clear flat-bottom 96-well plate.

2. For each reaction well, mix 93 µL Assay Buffer, 1 µL Dye Reagent and 1 µL Enzyme in a clean tube. Transfer 90 µL Working Reagent into each assay well. Tap plate to mix. Freeze unused reagents for future use.
3. Incubate 60 min at room temperature. Read optical density at 570nm (550-585nm).

Note: if the Sample OD is higher than the Standard OD at 1 mM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

CALCULATION

Subtract blank OD (water, #4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The sialic acid concentration of a Sample is calculated as

$$\text{[Sialic Acid]} = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \times n \quad \text{(mM)}$$

where OD_{SAMPLE} and OD_{H2O} are the optical density values of the sample and water, Slope is the slope of the standard curve in mM^{-1} and *n* is the dilution factor of the sample (*n* = 6 for hydrolyzed samples and *n* = 1 for free Sialic Acid samples).

Conversions: 1 mM NANA equals 30.9 mg/dL or 309 ppm.

FLUORIMETRIC PROCEDURE

1. For fluorimetric assays, the linear detection range is 2 to 100 µM sialic acid. Dilute the Standards prepared in Colorimetric Procedure 1:10 in H_2O. Transfer 10 µL standards and 10 µL samples into separate wells of a *black* 96-well plate.
2. Add 90 µL Working Reagent (see *Colorimetric Procedure*). Tap plate to mix.
3. Incubate 60 min at room temperature and read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

If assays in 384-well plate are desired, use 5µL Standards and 45 µL Working Reagent.

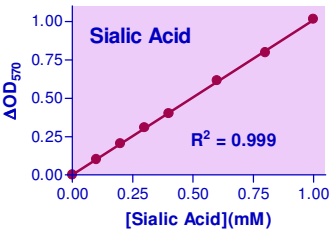
The sialic acid concentration of a Sample is calculated as

$$\text{[Sialic Acid]} = \frac{F_{SAMPLE} - F_{H2O}}{\text{Slope}} \times n \quad \text{(µM)}$$

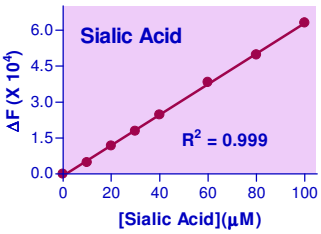
where F_{SAMPLE} and F_{H2O} are the fluorescence values of the sample and water, Slope is the slope of the standard curve in μM^{-1} and *n* is the dilution factor of the sample (*n* = 6 for hydrolyzed samples and *n* = 1 for free Sialic Acid samples).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, Clear flat-bottom 96-well plates, black 96-well or 384-well plates (e.g. Corning Costar) and plate reader.



96-well colorimetric assay



96-well fluorimetric standard

PUBLICATIONS

1. Cadaoas, J., et al. (2021). Galactosialidosis: Preclinical enzyme replacement therapy in a mouse model of the disease, a proof of concept. Molecular Therapy - Methods & Clinical Development. 20: 191-203.
2. Ha, T. K., et al. (2020). Knockout of sialidase and pro-apoptotic genes in Chinese hamster ovary cells enables the production of recombinant human erythropoietin in fed-batch cultures. Metabolic Engineering. 57: 182-192.