

## 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  $\lambda_{em}/\lambda_{ex} = 585/530nm$  is directly proportional to sialic acid concentration in the sample.

#### KEY FEATURES

**Sensitive and accurate.** Use as little as 10  $\mu L$  samples. Linear detection range in 96-well plate: 0.02 to 1 mM sialic acid for colorimetric assays and 2 to 100  $\mu M$  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

<b>Assay Buffer:</b> 10 mL	<b>Hydrolysis Reagent:</b> 10 mL
<b>Enzyme:</b> 120 $\mu L$	<b>Neutralization Reagent:</b> 5 mL
<b>Dye Reagent:</b> 120 $\mu L$	<b>Standard:</b> 500 $\mu L$ 10 mM Sialic Acid

**Storage conditions.** The kit is shipped on dry ice. Store all reagents at -20°C. Shelf life of three 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.

- Combine 20  $\mu L$  of sample with 80  $\mu L$  Hydrolysis Reagent in a microcentrifuge tube (screw cap tube is preferable) and incubate at 80°C for 60 min.
- Allow sample to cool to room temperature and briefly centrifuge at 14000 rpm to spin down the hydrolysis mixture.
- Add 20  $\mu L$  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.

#### COLORIMETRIC PROCEDURE

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

- Equilibrate all components to room temperature. Prepare a 1 mM Standard Premix by mixing 50  $\mu L$  of the 10 mM Standard and 450  $\mu L$  dH<sub>2</sub>O. Dilute Standard in distilled water as follows.

No	Premix + H <sub>2</sub> O	Vol ( $\mu L$ )	Sialic Acid (mM)
1	100 $\mu L$ + 0 $\mu L$	100	1.0
2	60 $\mu L$ + 40 $\mu L$	100	0.6
3	30 $\mu L$ + 70 $\mu L$	100	0.3
4	0 $\mu L$ + 100 $\mu L$	100	0

Transfer 10  $\mu L$  standards and 10  $\mu L$  samples into separate wells of a clear flat-bottom 96-well plate.

- For each reaction well, mix 93  $\mu L$  Assay Buffer, 1  $\mu L$  Dye Reagent and 1  $\mu L$  Enzyme in a clean tube. Transfer 90  $\mu L$  Working Reagent

into each assay well. Tap plate to mix. Freeze unused reagents for future use.

- 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

$$[\text{Sialic Acid}] = \frac{OD_{\text{SAMPLE}} - OD_{\text{H}_2\text{O}}}{\text{Slope}} \times n \text{ (mM)}$$

where OD<sub>SAMPLE</sub> and OD<sub>H2O</sub> are the optical density values of the sample and water, Slope is the slope of the standard curve in mM<sup>-1</sup> 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

- For fluorimetric assays, the linear detection range is 2 to 100  $\mu M$  sialic acid. Dilute the Standards prepared in Colorimetric Procedure 1:10 in H<sub>2</sub>O. Transfer 10  $\mu L$  standards and 10  $\mu L$  samples into separate wells of a black 96-well plate.
- Add 90  $\mu L$  Working Reagent (see *Colorimetric Procedure*). Tap plate to mix.
- Incubate 60 min at room temperature and read fluorescence at  $\lambda_{ex} = 530nm$  and  $\lambda_{em} = 585nm$ .

If assays in 384-well plate are desired, use 5 $\mu L$  Standards and 45  $\mu L$  Working Reagent.

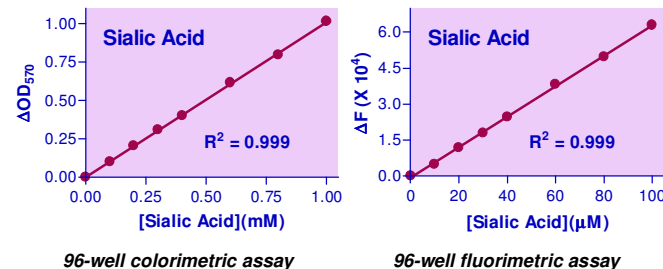
The sialic acid concentration of a Sample is calculated as

$$[\text{Sialic Acid}] = \frac{F_{\text{SAMPLE}} - F_{\text{H}_2\text{O}}}{\text{Slope}} \times n \text{ (}\mu\text{M)}$$

where F<sub>SAMPLE</sub> and F<sub>H2O</sub> are the fluorescence values of the sample and water, Slope is the slope of the standard curve in  $\mu M^{-1}$  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.



#### LITERATURE

- Sugahara, K. et al. (1980). Enzymatic Assay of Serum Sialic Acid. Clinica Chimica Acta 108:493-8.
- Simpson, H. et al. (1993). Serum sialic acid enzymatic assay based on microtitre plates: application for measuring capillary serum sialic acid concentrations. Br J Biomed Sci. 50:164-7.