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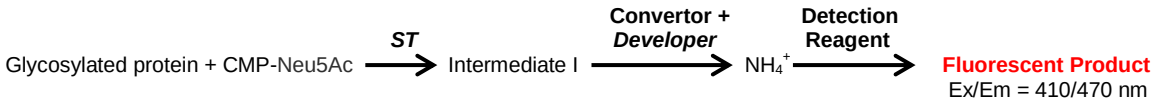
# Sialyltransferase Activity Assay Kit (Fluorometric)

*06/20*

**(Catalog # K2048-100; 100 assays; Store at -20ºC)**

## I. Introduction:

Sialyltransferases (ST, Galactosyldiacylglycerol alpha-2,3-sialyltransferase; 2.4.99.5) belong to the glycosyltransferase family and are expressed in a variety of organisms from bacteria to higher mammals. Previously, it was believed to be not expressed in plants, but sialyltransferase like proteins have been recently identified in rice. STs catalyze the transfer of sialic acid (a kind of N-acetylneuraminic acid) from CMP-Neu5Ac moieties to sugar molecules on the terminal regions of glycolipids and glycoproteins. In higher animals, terminal sialic acid residue-containing glycoconjugates have various important functions including modulation of the immune system, brain development, etc. In bacteria, they play a role in virulence and are present on the bacterial surface to protect them from the immune system. **BioVision's Sialyltransferase Activity Assay Kit** is a simple, two-step plate based assay that measures the fluorescent product at Ex/Em = 410/470 nm. The assay consists of three enzymatic steps leading to the generation of ammonia. Ammonia then reacts with the detection reagent to form a fluorescent product measured at Ex/Em = 410/470 nm. The kit can detect activity from both α-1,6 and α-2,6 sialyltransferases and can measure as low as 20 µU ST activity under assay conditions.




## II. Application:

Measurement of Sialyltransferase activity using a 96-well plate format

## III. Sample Types:

- Tissue or cell lysate
- Recombinant enzyme
- Purified protein

## IV. Kit Contents:

| Components | K2048-100 | Cap Code | Part Number |
|---|---|---|---|
| ST Assay Buffer | 25 ml | WM | K2048-100-1 |
| ST Substrate I | 1 vial | Red | K2048-100-2 |
| ST Substrate II | 1 vial | Blue | K2048-100-3 |
| ST Convertor | 1 vial | Green | K2048-100-4 |
| ST Developer | 1 vial | Orange | K2048-100-5 |
| ST Detection Reagent | 1.2 ml | Amber | K2048-100-6 |
| ST Positive Control | 1 vial | Purple | K2048-100-7 |
| $NH_4Cl$ Standard | 100 µl | Yellow | K2048-100-8 |
| Microplate Sealing Film | 1 | --- | K2048-100-9 |

## V. User Supplied Reagents and Equipment:

- $dH_2O$
- ß-mercaptoethanol
- 100% Ethanol
- 96-well black plate with flat bottom
- Multi-well spectrophotometer
- 10 kDa spin column (BioVision Cat# 1997)

## VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay. Components are stable for at least twelve months.

- **ST Assay Buffer & $NH_4Cl$ Standard (100 mM):** Warm to room temperature (RT) before use.
- **ST Substrate I & ST Substrate II:** Reconstitute each vial in 220 µl $dH_2O$ Divide into aliquots and store at -20°C.
- **ST Convertor & ST Developer:** Reconstitute each vial in 220 µl ST Assay Buffer. Divide into aliquots and store at -80°C. Keep on ice when in use**.** Avoid repeated freeze-thaw cycles.
- **ST Positive Control:** Reconstitute the vial in 22 µl ST Assay Buffer. Divide into aliquots and store at -20ºC. Keep on ice when in use. Avoid repeated freeze-thaw cycles.
- **ST Detection Reagent:** Store at -20°C. Keep on ice when in use.

## VII. Sialyltransferase Activity Assay Protocol:

1. **Sample Preparation:** Homogenize cells (4 x $10^5$ cells) or tissue (20 mg) with 100 µl ST Assay buffer to perform lysis and keep on ice for 10 min followed by centrifugation at 10,000 *x g* and 4ºC for 15 min. Collect the supernatant (lysate) and estimate the protein concentration using any preferred method. We recommend BCA protein assay kit (BioVision Cat# K813-2500). Protein concentration should range between 5 and 20 µg/µl. Dilute the lysate (if needed) using with $dH_2O$ water. In order to get rid of any interfering small molecules, dilute the lysate with $dH_2O$ 5-10 times and filter through 10 kDa spin columns (BioVision Cat# 1997). Repeat the ultrafiltration step twice. Small molecules will be removed in the ultrafiltrate, and the **ultraconcentrate** should be used for the ST activity assay.

Prepare two wells for each sample type labeled as **"Sample Background Control"** (SBC), and **"Sample"** (S). Add 2-8 µl sample (up to 40 µg protein) into each pair of wells of a 96-well black plate. For **Positive Control**, add 4 µl of the reconstituted ST Positive Control into the desired well(s). Adjust volume in each well to 50 µl with ST Assay Buffer. For **Substrate Background Control**, add 50 µl of ST Assay Buffer to a well.

**Notes:**

**a)** We recommend using freshly prepared samples for activity analysis immediately.
**b)** For Unknown Samples, we suggest testing several concentrations to ensure that the readings are within the Standard Curve range.
**c)** Perfused tissue should be used as hemoglobin interferes with the assay.

2. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 50 µl Mix containing:

| | Reaction Mix | Background Mix |
|---|---|---|
| ST Assay Buffer | 42 µl | 44 µl |
| ST Substrate I | 2 µl | 2 µl |
| ST Substrate II | 2 µl | -- |
| ST Convertor | 2 µl | 2 µl |
| ST Developer | 2 µl | 2 µl |

Mix well and add 50 µl Reaction Mix to wells containing Sample, Substrate Background Control and Positive Control. Add 50 µl Background Mix to SBC wells. Mix well**.**

3. **$NH_4Cl$ Standard Curve:** Dilute the $NH_4Cl$ Standard (100 mM) stock to 500 $\mu$M $NH_4Cl$ Standard working solution by adding 5.0 µl of 100 mM $NH_4Cl$ Standard stock into 995 µl of $dH_2O$. Add 0, 2, 4, 6, 8, 10 µl of 500 µM $NH_4Cl$ Standard working solution into a series of well in a 96-well black plate to generate 0, 1, 2, 3, 4, 5 nmoles of $NH_4Cl$ Standard/well respectively. Adjust the volume of each well to 100 µl with ST Assay Buffer. **Note:** Ammonia present in the air can result in high background.
4. **Incubation:** Cover the plate with the provided Microplate sealing film and incubate at 37°C for 30 min.
5. **Ammonia Detection Mix Preparation:** Prepare ß-mercaptoethanol (ß-Me) working solution by mixing 11 µl of 14.3 M ß-Me with 1989 µl of 100% Ethanol and keep on ice. Prepare the Ammonia Detection Mix as follows:

| | Ammonia Detection Mix |
|---|---|
| ST Buffer | 86 µl |
| ST Detection Reagent | 7 µl |
| ß-Me working solution | 7 µl |

Add 100 µl of Ammonia Detection Mix to all wells including Standards, Samples, Sample Background Controls, Substrate Background Control, and Positive Control and incubate at 37°C for 45 min.

**Note:** ß-Me working solution should always be made fresh and kept on ice.

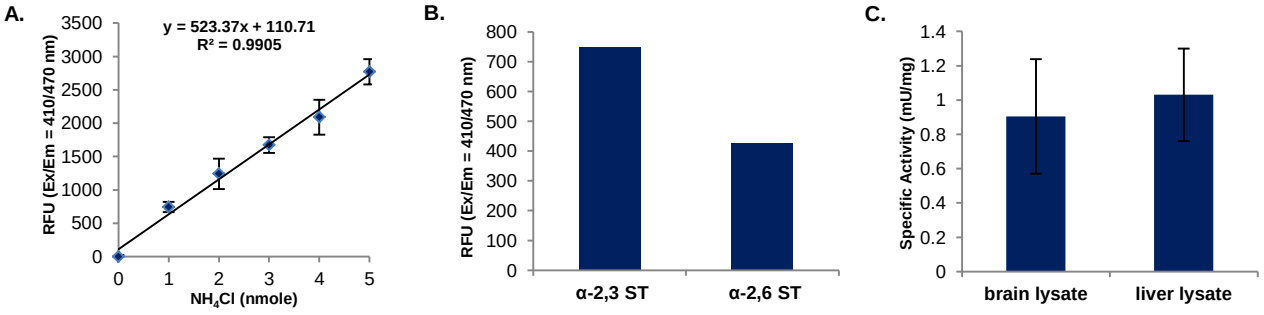
6. **Measurement:** Record Fluorescence of all wells at Ex/Em = 410/470 nm after 45 min in end point mode.
7. **Calculation:** Subtract the 0 Standard reading from all Standard readings and SBC reading from Sample readings respectively. If the Substrate Background Control reading is higher than the SBC reading, subtract its value instead. Plot the $NH_4Cl$ Standard Curve. Apply the corrected Sample readings to the $NH_4Cl$ Standard Curve to get nmol of $NH_4^+$ in samples.

Calculate the Sialyltransferase activity of the samples as:

**Sample Sialyltransferase Activity = B / (Δt X p) (nmol / (min X mg)) = mU/mg**

Where: **B** = $NH_4^+$ in sample(s) (nmol).
**Δt** = reaction time (30 min)
**p** = sample protein content added (mg)

**Unit Definition**: One unit of Sialyltransferase is the amount of enzyme leads to the generation of 1.0 µmol of $NH_4^+$ per minute at pH 7 at 37°C.




**Figures: A).** $NH_4Cl$ Standard Curve**. B).** Fluorescence signal obtained for α-2,3 and α-2,6 ST sialyltransferases**. C).** ST specific activity in brain and liver lysates. Experiments were conducted according to kit protocol.

## VIII. Related Products:

Sialic Acid (NANA) Colorimetric/Fluorometric Assay Kit (K566)
Neuraminidase Activity Fluorometric Assay Kit (K732)
UGT Activity Assay / Ligand Screening Kit (Fluorometric) (K692)
Anti- CDw75 Antibody (LN-1) (A1573)

***FOR RESEARCH USE ONLY! Not to be used on humans.***