ab282920 – Sialyltransferase Activity Assay Kit (Fluorometric)

For the measurement of Sialyltransferase activity using a 96-well plate format.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab282920

Storage and Stability

Entire assay kit should be stored at -20°C, protected from light. The components are stable for one year when stored as recommended.

Materials Supplied

Item	Quantity	Storage Condition
ST Assay Buffer	25 mL	-20°C
ST Substrate I	1 vial	-20°C
ST Substrate II	1 vial	-20°C
ST convertor	1 vial	-20°C
ST developer	1 vial	-20°C
ST detection Reagent	1.2 mL	-20°C
ST positive control	1 vial	-20°C
NH4Cl Standard	100 µl	-20°C
Microplate Sealing Film	1	-20°C

Materials Required, Not Supplied

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

- dH2O
- ß-mercaptoethanol
- 100% Ethanol
- 96-well black plate with flat bottom
- Multi-well spectrophotometer
- 10 kDa spin column (ab93349)

Reagent Preparation Before using the kit, spin the tubes prior to opening.
ST Assay Buffer & NH4Cl Standard (100 mM): Warm to room temperature (RT) before use.
ST Substrate I & ST Substrate II: Reconstitute each vial in 220 µl dH2O 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 -20°C. Keep on ice when in use. Avoid repeated freeze-thaw cycles.
ST Detection Reagent: Store at -20°C. Keep on ice when in use.

Sialyltransferase Activity Assay Protocol:

Sample Preparation

1. Homogenize cells (4 x 105 cells) or tissue (20 mg) with 100 µl ST Assay buffer to perform lysis & keep on ice for 10 min followed by centrifugation at 10,000 x g and 4°C for 15 min.
2. Collect the supernatant (lysate) and estimate the protein concentration using any preferred method. Protein concentration should range between 5 and 20 µg/µl.
3. Dilute the lysate (if needed) using with dH2O water.
4. In order to get rid of any interfering small molecules, dilute the lysate with dH2O 5-10 times and filter through 10 kDa spin columns.
5. Repeat the ultrafiltration step twice.
6. Small molecules will be removed in the ultrafiltrate, and the ultraconcentrate should be used for the ST activity assay.
7. 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.
8. 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.
9. 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

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:

Item	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.

NH4Cl Standard Curve:

1. Dilute the NH4Cl Standard (100 mM) stock to 500 µM NH4Cl Standard working solution by adding 5.0 µl of 100 mM NH4Cl Standard stock into 995 µl of dH2O.
2. Add 0, 2, 4, 6, 8, 10 µl of 500 µM NH4Cl Standard working solution into a series of well in a 96-well black plate to generate 0, 1, 2, 3, 4, 5 nmoles of NH4Cl Standard/well respectively.
3. Adjust the volume of each well to 100 µl with ST Assay Buffer.

Note: Ammonia present in the air can result in high background.

Incubation
Cover the plate with the provided Microplate sealing film and incubate at 37°C for 30 min.

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:

Item	Reaction 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.

Measurement
Record Fluorescence of all wells at Ex/Em = 410/470 nm after 45 min in end point mode.

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 NH4Cl Standard Curve.
Apply the corrected Sample readings to the NH4Cl Standard Curve to get nmol of NH4 + in samples.
Calculate the Sialyltransferase activity of the samples as:

$$\textbf{Sample Sialyltransferase Activity} = B / (\Delta t \times p) \text{ (nmol / (min X mg))} = \text{mU/mg}$$

Where:
B = NH4+ 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 NH4 + per minute at pH 7 at 37°C

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)

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