α(2-3)-Sialyltransferase

[β-D-galactosyl(1-4)N-acetyl-β-D-glucosamine-α(2-3)N-acetylneuraminyltransferase: ST3Gal6]

SPECIFICATIONS

Product Code: GKT-S23
Activity: ≥80 U/µg
Storage: -20°C
Shipped on ice pack for next day delivery.
Formulation: 50 mM MES, 200 mM NaCl (pH 6.4)

α(2-3)-Sialyltransferase [β-D-galactosyl(1-4)N-acetyl-β-D-glucosamine-α(2-3)N-acetyl neuraminyltransferase: Beta-galactoside alpha-2,3-sialyltransferase, EC 2.4.99.4] is recombinant from a human gene and expressed in HEK 293F cells.

α(2-3)-Sialyltransferase (ST3Gal6) transfers sialic acid from a donor substrate (CMP-NANA) to Galβ(1-4) GlcNAc units on glycoproteins and complex molecules.

The α(2-3) sialic acid linkage is found on the N-glycans of glycoproteins produced in Chinese hamster ovary (CHO) cells [1]. N-glycans that are α(2-3)-sialylated are known to have a shorter hydrophilic interaction liquid chromatography (HILIC) retention time than isomeric glycans with α(2-6) sialic acid linkages [2]. Sialic acid linkage may also be determined by exoglycosidase digests with Sialidase S (GK80021), which releases non-reducing terminal α(2-3)-linked sialic acid, and Sialidase A (GK80040) which releases α(2-3,6,8,9)-linked sialic acid.

Applications:

- For in vitro sialylation of glycoproteins such as human antibodies.

PRODUCT DESCRIPTION

Supplied Reagents

WS0329 CMP-NANA
(3 x 10 mg; cytidine 5'-monophospho-N-acetylneuraminic acid disodium salt)

WS0346 5x Reaction Buffer
[1 ml; 1 M MES (pH 6.5)]

Molecular Weight: 34.5 kDa (by cDNA)

pH:

Recommended: 6.5

Stability: Store enzyme at -20°C. Avoid repeated freeze-thawing as this decreases the efficacy of the enzyme. Dispense working aliquots (50 µg) after initial thaw if not utilizing entire quantity.

ASSAY

One unit of α(2-3)-Sialyltransferase is defined as the amount of enzyme required to release one pmol of CMP from CMP-NANA (measured as inorganic phosphate released from CMP) per minute per µg (enzyme) at an enzyme concentration of 10 µg/ml at pH 7.5 and 37°C.

SUGGESTIONS FOR USE

Before use, briefly centrifuge the vial to ensure that all material is at the base of the vial. Ensure that reagents, substrates and laboratory-ware are free from contaminants and proteases.

The amount of enzyme required for sialylation and the amount of sialylation will vary depending on the target molecule or application.

The recommended incubation time is 2–6 hours. Longer incubation times (greater than 8 hours) may result in less efficient sialylation due to reverse kinetics. A time course to determine the optimal conditions for different intended targets is recommended.

The suggested buffer conditions for sialylation are 200 mM MES, pH 6.5 with 6 mM CMP-NANA as donor substrate.

Suggested Procedure for Sialylation

1. Prepare 1250 µl of 1x Reaction Buffer: add 250 µl of the supplied 5x Reaction Buffer to 1000 µl of ultrapure water. The resulting 1x buffer contains 200 mM MES, pH 6.5.

2. Prepare the target glycoprotein in 1x Reaction Buffer at concentration of 5–10 mg/ml (using either 1x or 5x reaction buffer, depending on the starting concentration of glycoprotein).

3. Dissolve 1 vial (10 mg) of CMP-NANA in 1000 µl of 1x Reaction Buffer.

4. Combine 300 µg (30–60 µl) of target glycoprotein with 150 µg (15 µl) CMP-NANA and 50 µg (10 µl) of α(2,3) Sialyltransferase.

5. To obtain a final reaction volume of 100 µl, add 1x Reaction Buffer if necessary.

6. Incubate at 37°C for 2–6 hours.

7. Stop the incubation by freezing at -15 to -25°C.

NOTE: To avoid the potential for reaction-related artifacts affecting downstream analysis (e.g., in vitro and in vivo biological assays, PK/PD studies, mass spectrometric methods, etc.), the removal of excess enzyme and/or CMP-NANA (e.g., by MWCO filtration, gel filtration, affinity chromatography, etc.) may be required.

REFERENCES

1. Lee EU, Roth J, Paulson JC. Alteration of terminal glycosylation sequences of N-linked oligosaccharides of

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2. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science. 2008 Apr 18;320(5874):373-6.

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