Recombinant Human SIAE His-tag

Catalog Number: 11497-SI

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human SIAE protein Ile24-Lys523, with a C-terminal 6-His tag Accession # Q9HAT2
N-terminal Sequence Analysis	Ile24
Predicted Molecular Mass	57 kD

SPECIFICATIONS

SDS-PAGE	63-69 & 88-97 kD, under reducing conditions.
Activity	Measured by its ability to hydrolyze p-nitrophenylacetate. The specific activity is >25000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 8.0
- Recombinant Human SIAE His tag (rhSIAE) (Catalog # 11497-SI)
- Substrate: 4-Nitrophenyl Acetate, 100 mM stock in acetone
- Clear 96 Well Plate
- Plate Reader with Absorbance Read Capability

Assay

1. Dilute rhSIAE to 1.2 µg/mL in Assay Buffer.
2. Dilute Substrate to 8 mM in Assay Buffer.
3. Load into a plate 50 µL of 1.2 µg/mL rhSIAE, and start the reaction by adding 50 µL of 8 mM Substrate. For Substrate Blank, load 50 µL of Assay Buffer and 50 µL of 8 mM Substrate.
4. Read plate at 410 nm in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 18500 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhSIAE: 0.06 µg
- Substrate: 4 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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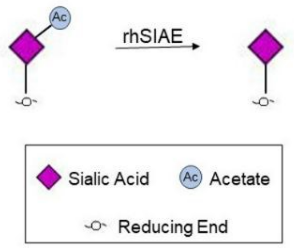
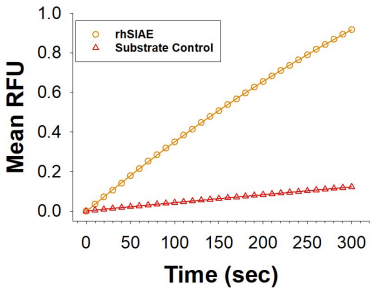
DATA

Enzyme Activity



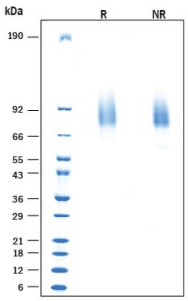
Recombinant Human SIAE His-tag Protein Enzyme Activity Diagram. Recombinant Human SIAE His-tag Protein (Catalog # 11497-SI) catalyzes the removal of O-acetyl ester groups from position 9 of Sialic Acid.

Enzyme Activity



Recombinant Human SIAE His-tag Protein Enzyme Activity Assay. Recombinant Human SIAE His-tag Protein (Catalog # 11497-SI) is measured by its ability to hydrolyze p-nitrophenylacetate.

SDS-PAGE



Recombinant Human SIAE His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human SIAE His-tag Protein (Catalog # 11497-SI) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 63-69 & 88-97 kDa, under reducing conditions.

BACKGROUND

Recombinant human Sialate O-acetylesterase (SIAE) is a glycosylated lysosomal protein that has two transcript isoforms: the cytosolic sialic acid esterase (Cse) that lacks 35 amino acids at the N-terminus and the lysosomal/membrane-associated sialic acid esterase (Lse) (1, 2). The Lse form is the predominantly expressed form responsible for the acetyl esterase activity on NeuAc in most tissues (2) and represents the recombinant product form. SIAE contains a signal sequence, a variable N-terminus, and a core protein structure that is unique within the SGNH hydrolase family (2) and contains a shallow active site pocket that accommodates binding of various complex substrates with acetylated sialic acid. SIAE Lse form catalyzes the removal of O-acetyl ester groups from position 9 of Sialic acid and is highly expressed in the testis, colon, and prostrate (3). SIAE deficiency has been shown to impact B cell development, signaling, and immunological tolerance through modulation of Siglec binding (4). Many SIAE variants in humans that result in defective activity or improper secretion have been identified in patients with autoimmune diseases including Crohn's disease, Sjogren's syndrome, lupus, rheumatoid arthritis, and type I diabetes (5). In addition, SIAE modulation of Siglec binding and sialic acid levels may play a key role in the immune evasion pathway associated with cancer (6). SIAE has also been implicated to play an important role in immune modulation during pregnancy (7).

References:

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