Recombinant Human Protein Disulfide Isomerase/P4HB

Catalog Number: 4236-DI

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Asp18-Lys505, with a C-terminal 10-His tag Accession # P07237
N-terminal Sequence Analysis	Asp18
Predicted Molecular Mass	56 kDa

SPECIFICATIONS

SDS-PAGE	60 kDa, reducing conditions
Activity	Measured by its ability to promote aggregation of insulin in the presence of DTT. The specific activity is >7.5 A_{650}/cm/min/mg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>80%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Buffer A: 100 mM NaH_2PO_4, 3 mM DTT, pH 7.0
- Buffer B: 100 mM NaH_2PO_4, 3 mM EDTA, pH 7.0
- Recombinant Human Protein Disulfide Isomerase/P4HB (rhP4HB) (Catalog # 4236-DI)
- Insulin (Sigma, Catalog # I-5500), 10 mg/mL solution in 50 mM Tris, pH 7.5 (solution will be cloudy)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute insulin to 1.5 mg/mL in Buffer B.
2. Incubate diluted insulin for 10 minutes at room temperature (insulin will solubilize).
3. Dilute rhP4HB to 45 µg/mL in Buffer A.
4. Mix 100 µL of 45 µg/mL rhP4HB and 200 µL of 1.5 mg/mL insulin. Create a Substrate Blank with 100 µL Buffer A and 200 µL insulin.
5. Incubate at room temperature for 20 minutes.
6. Load in a clear 96-well plate 100 µL from each reaction vial in duplicate.
7. Read at 650 nm (absorbance) in kinetic mode for 5 minutes.
8. Calculate specific activity:

$$\text{Specific Activity (Abs/cm/min/mg)} = \frac{\text{Adjusted } V_{max}\text{* (Abs/min)**}}{\text{path corr.(cm)*** x amount of enzyme (mg)}}$$

*Adjusted for Substrate Blank
**Note: the output of many spectrophotometers in kinetic mode is in mOD.
***Using the pathlength correction of 0.32 cm

Final Assay Conditions

Per Well:

- rhP4HB: 0.0015 mg
- Insulin: 0.100 mg

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.

BACKGROUND

Protein Disulfide Isomerase, also known as prolyl 4-hydroxylase subunit beta (P4HB), protocollagen hydroxylase, cellular thyroid hormone binding protein p55 and glutathione-insulin transhydrogenase (1-3) is an abundant multifunctional enzyme that belongs to the Protein Disulfide Isomerase family. It contains two thioredoxin domains that catalyze the formation, breakage and rearrangement of disulfide bonds. When present as a tetramer consisting of two alpha subunits and two beta subunits, this enzyme functions as a hydroxylase catalyzing the hydroxylation of prolyl residues in preprocollagen. P4HB has various additional functions (4-7). It binds thyroid hormone. It acts as a chaperone that inhibits aggregation of misfolded proteins. It plays a role in both the influx and efflux of S-nitrosothiol-bound nitric oxide. It is also a subunit of the microsomal triglyceride transfer protein complex.

References:

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