Recombinant Human TIGAR/C12orf5 His-tag

Catalog Number: 11008-TG

DESCRIPTION

Source	*E. coli*-derived human TIGAR/C12orf5 protein Ala2-Arg270 with a C-terminal 6-His tag Accession # Q9NQ88.1
N-terminal Sequence Analysis	Ala2
Predicted Molecular Mass	31 kDa

SPECIFICATIONS

SDS-PAGE	27-30 kDa, under reducing conditions
Activity	Measured by its ability to cleave a substrate, p-Nitrophenyl phosphate (pNPP). The specific activity is >10 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.5
- Recombinant Human TIGAR/C12orf5 (rhTIGAR) (Catalog # 11008-TG)
- Substrate: 4-Nitrophenyl Phosphate (Sigma, Catalog # N2765), 10 mM stock in deionized water
- Sodium Hydroxide (NaOH) (Sigma, Catalog # 221465), 2 M stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhTIGAR to 40 µg/mL in Assay Buffer.
2. Dilute Substrate to 5 mM in Assay Buffer.
3. Dilute 2 M NaOH to 0.2 M NaOH in deionized water.
4. Prepare reaction mixtures by combining equivalent volumes of dilute rhTIGAR and dilute Substrate in microtubes. Include an Enzyme Control by combining dilute rhTIGAR with twice the volume of 0.2 M NaOH, mix briefly, then add a volume of dilute Substrate equivalent to the volume of rhTIGAR. The Enzyme Control will have 2x the volume of the reaction mixture.
5. Incubate Reactions and Enzyme Controls at 37 °C for 3 hours.
6. Load 100 µL of Reactions into a plate and stop the reactions by adding 100 µL of 0.2 M NaOH.
7. Load 200 µL of Enzyme Controls into plate.
8. Read plate at 410 (absorbance) in endpoint mode.
9. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Abs* (OD) x Conversion Factor** (pmol/OD)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Enzyme Controls
**Derived using calibration standard 4-Nitrophenol (Sigma, Catalog # 241326)

Final Assay Conditions

Per Well:

- rhTIGAR: 2 µg
- Substrate: 1.25 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.

DATA

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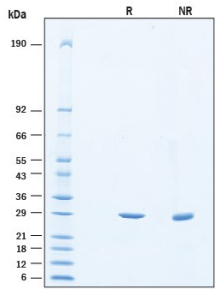
SDS-PAGE



Recombinant Human TIGAR/C12orf5 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human TIGAR/C12orf5 His-tag Protein (Catalog # 11008-TG) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 27-30 kDa.

BACKGROUND

Fructose-2,6-bisphosphatase TIGAR (TIGAR), also known as TP53-induced glycolysis and apoptosis regulator, is a highly conserved, ubiquitously expressed enzyme from the phosphoglycerate mutase family that hydrolyzes fructose-2,6-bisphosphate, a key regulator of cellular metabolism that activates glycolysis and inhibits gluconeogenesis (1). Consequently, TIGAR acts as a negative regulator of glycolysis and its activity results in pentose phosphate pathway activation and NADPH production. Human TIGAR is a 270 amino acid monomeric protein with a histidine phosphatase fold containing a phosphate coordinated to a catalytic histidine in the active site (2). It is located both in the cytoplasm and in organelles where its function may vary (1); translocation of TIGAR to the nucleus promotes cell survival during chemotherapy and hypoxia (3) while transportation to the mitochondria under hypoxia can enhance HK2 activity and maintain cell survival (4) or interact with ATP5A1 to reduce oxidative stress (1). TIGAR is a target gene of p53 through binding sequences in the promoter region (5) and may promote cancer metastasis through both enzymatic function and through interactions that allow it to mediate signaling pathways that inhibit autophagy and apoptosis (6, 7). TIGAR is highly expressed in some tumors (1) and may be a potential therapeutic target for cancers (6-8), cardiovascular disease (9), and neurological diseases (10-13).

References:

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