R&D SYSTEMS
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# Recombinant Human PHGDH His-tag

Catalog Number: 10131-DH

## DESCRIPTION

| | |
|---|---|
| **Source** | *E. coli*-derived human PHGDH protein<br>Ala2-Phe533<br>Accession # O43175-1<br>with a C-terminal 6-His tag |
| **N-terminal Sequence Analysis** | Ala2 |
| **Predicted Molecular Mass** | 57 kDa |

## SPECIFICATIONS

| | |
|---|---|
| **SDS-PAGE** | 57 kDa, under reducing conditions |
| **Activity** | Measured by the ability to catalyze the oxidation of 3-phospho-D-glycerate.<br>The specific activity is >350 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, Glycerol and TCEP. See Certificate of Analysis for details. |

## Activity Assay Protocol

**Materials**

- Assay Buffer: 50 mM Tris, 800 mM NaCl, 0.2 mM DTT, pH 9.0
- Recombinant Human Phosphoglycerate Dehydrogenase (rhPHGDH) (Catalog # 10131-DH)
- 3-Phospho-D-Glyceric Acid (PGA) (Sigma, Catalog # P8877), 200 mM stock in deionized water
- β-Nicotinamide Adenine Dinucleotide (β-NAD) (Sigma, Catalog # N6522), 100 mM stock in deionized water
- UV Plate (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Dilute rhPHGDH to 5 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 20 mM PGA and 4 mM β-NAD in Assay Buffer.
3. Load into a plate 50 µL of 5 µg/mL rhPHGDH, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Read plate at 340 nm (absorbance) 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 extinction coefficient 6220 $M^{-1}cm^{-1}$.
***Using the path correction 0.32 cm.

**Final Assay Conditions**

Per Well:

- rhPHGDH: 0.25 µg
- PGA: 10 mM
- β-NAD: 2 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

## DATA



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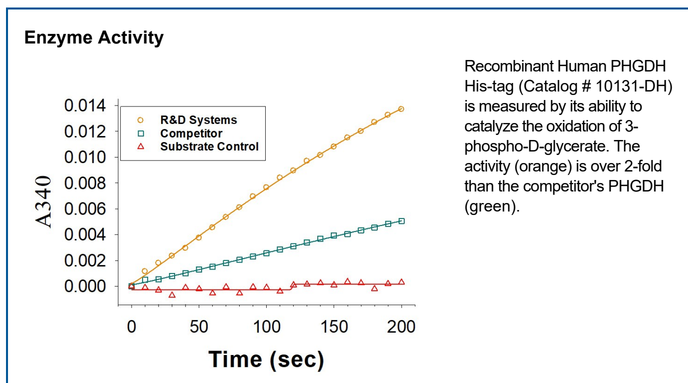
# Recombinant Human PHGDH His-tag

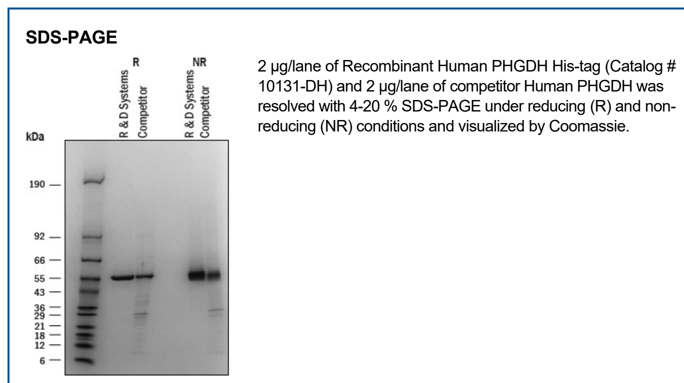
Catalog Number: 10131-DH

## Enzyme Activity




Recombinant Human PHGDH His-tag (Catalog # 10131-DH) is measured by its ability to catalyze the oxidation of 3-phospho-D-glycerate. The activity (orange) is over 2-fold than the competitor's PHGDH (green).

## SDS-PAGE




2 μg/lane of Recombinant Human PHGDH His-tag (Catalog # 10131-DH) and 2 μg/lane of competitor Human PHGDH was resolved with 4-20 % SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie.

## BACKGROUND

Phosphoglycerate dehydrogenase (PHGDH) catalyzes the oxidation of 3-phospho-D-glycerate to 3-phosphooxypyruvate, which is the first step of the L-serine biosynthesis pathway. Human PHGDH is a Type I NADH-dependent enzyme that forms an active oligomer where each monomer is composed of 4 domains: a substrate-binding domain, a nucleotide-binding domain, and two regulatory domains: ASB (allosteric substrate binding) and ACT (Aspartate kinase, Chorismate mutase, and Tyr A)(1). Serine biosynthesis by PHGDH is the sole source of serine biosynthesis in mammals (2) and conditional knockout confirmed L-serine synthesis by PHGDH is the source of D-serine in the brain (3). PHGDH null mice are embryonic lethal (4) and mutations that lead to PHGDH deficiency have been reported in infantile, juvenile and adult onset phenotypes (5, 6). PHGDH is also suggested to play a role in several cancers including breast (7), cervical (8), melanoma (9), colon (10), pancreatic (11), liver (12), and kidney (13) through increased PHGDH expression and enhanced tumor cell proliferation. Levels of PHGDH correlate with patient survival and may be used as a prognostic factor (14). A mutation resulting in inactive PHGDH (15), cell line knockdown (8, 11), and treatment with enzymatic inhibitors in cancer cells (13) result in decreased proliferation. Given the correlation between high PHGDH and cancer and the results from indirect inhibition of PHGDH activity, PHGDH is a pharmaceutical target for cancer therapy (16-18).

**References:**

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