Recombinant Human HPRT His-tag

Catalog Number: 10314-HP

DESCRIPTION

Source	*E. coli*-derived human HPRT protein Ala2-Ala218 Accession # P00492 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	25 kDa

SPECIFICATIONS

SDS-PAGE	27-29 kDa, under reducing conditions
Activity	Measured by its ability to produce GMP from guanine and phosphoribosyl pyrophosphate. The specific activity is >9000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 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: 0.1 M Tris, 100 mM $MgCl_2$, pH 8.5
- Recombinant Human HPRT His-tag (rhHPRT) (Catalog # 10314-HP)
- 5-phospho-D-ribose 1-diphosphate pentasodium salt (PRPP) (Sigma, Catalog # P8296), 50 mM stock in deionize water
- Guanine (Sigma, Catalog # G11950), 2 mM stock in deionized water with addition of 10 mM NaOH (or until pH of ~12) to solubilize
- UV Plate (Corning, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhHPRT to 0.4 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 2 mM PRPP and 120 µM Guanine in Assay Buffer.
3. Load 50 µL of 0.4 µg/mL rhHPRT into plate, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of Substrate Mixture.
4. Read plate at an absorbance of 257 nm in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Using the extinction coefficient 5817 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhHPRT: 0.020 µg
- PRPP: 1 mM
- Guanine: 60 µM

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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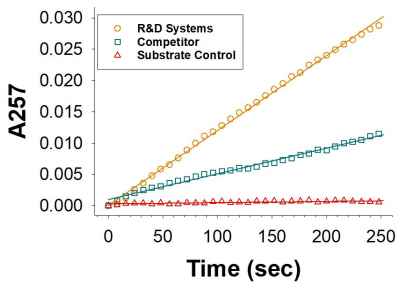
Recombinant Human HPRT His-tag

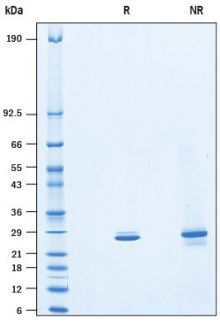
Catalog Number: 10314-HP

Enzyme Activity



Recombinant Human HPRT Hig-tag (Catalog # 10314-HP) is measured by its ability to produce GMP from guanine and phosphoribosyl pyrophosphate. The activity (orange) is over 2-fold higher than the competitor's HPRT (blue).

SDS-PAGE



2 µg/lane of Recombinant Human HPRT His-tag (Catalog # 10314-HP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 27-29 kDa under reducing conditions.

BACKGROUND

Hypoxanthine-guanine phosphoribosyltransferase (HPRT), a member of the phosphoribosyltransferase family, is a magnesium-dependent, cytoplasmic, ubiquitously-expressed (1) salvage enzyme involved in the primary pathway utilized for purine synthesis (2, 3). It catalyzes the transfer of phosphoribose from phosphoribosyl pyrophosphate to hypoxanthine and guanine bases to form inosine and guanine, respectively. Human HPRT forms a homotetramer of identical subunits that form dimer pairs (4, 5). Each 49 kDa subunit is 217 amino acids and consists of a core domain containing the active site and a hood domain (4, 5). HPRT deficiency in the salvage pathway leads to overproduction of purine, indicating it may play a role in regulation of purine production (6). Deficiency from multiple characterized single point mutations (7,8) causes hyperuricemia resulting in gout-like symptoms of Kelley-Seegmiller syndrome or if completely lacking in HPRT, a severe X-linked hereditary disorder, Lesch-Nyhan Disease, that is also characterized to cause significant neurological impairment (3, 6) by affecting multiple signaling pathways (9) and through dysregulation of cellular functions including proliferation, RNA metabolism, DNA replication and protein synthesis (3, 10). Given its ability to modulate cellular functions through purine regulation, HPRT is implicated to play a role in cancer. Significant correlation between HPRT mutations and increased cancer risk have been reported (11, 12). HPRT has been found surface-expressed in cancer (13) and thus has been proposed as a potential marker and therapeutic target (12).

References:

1. Melton, D. W. *et al.* (1986) Cell **44**:319.
2. Stout, J. T. *et al.* (1985) Ann. Rev. Genet. **19**:127.
3. Fasullo, M. and L. Endres. (2015) Int. J. Mol. Sci. **16**:9431.
4. Eads, J. C. *et al.* (1994) Cell 78:325.
5. Keough, D.T. *et al.* (2005) J. Mol. Biol. **351**:170.
6. Garcia-Gil, M. *et al.* (2018) Int. J. Mol. Sci. **19**: E3598.
7. Torres, R. J. and J.G. Puig (2007) Orphanet J. Rare Dis. **2**:48.
8. Zoref-Shani, E. *et al.* (2000) BBA Mol. Basic Dis. **1500**:197.
9. Guibinga, G. H. *et al.* (2014) PLoS ONE **9**:e96575.
10. Kang. T. H. *et al.* (2013 PLoS ONE **8**:e74967.
11. Townsend, M. H. *et al.* (2017) Cancer Clin. Oncol. **6**:19.
12. Townsend, M. H. *et al.* (2018) Med. Oncol. **35**:89.
13. Townsend, M. H. *et al.* (2017) OncoTargets Ther. **10**:1921.

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