

Human Erythropoietin (hEPO)



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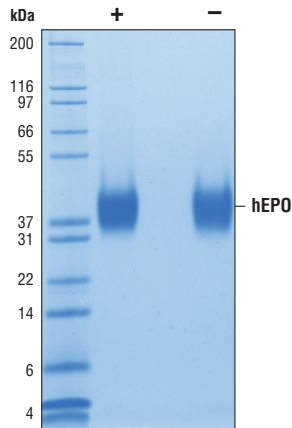
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Source: Recombinant human Erythropoietin (hEPO) Ala28-Arg193 (Accession #NP_000790) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hEPO contains no "tags" and the nonglycosylated protein has a calculated MW of 20,417. DTT-reduced and non-reduced protein migrate as 34-40 kDa polypeptides. Lower mobility in SDS-PAGE is due to glycosylation. The expected amino-terminal APPRL of recombinant hEPO was verified by amino acid sequencing.

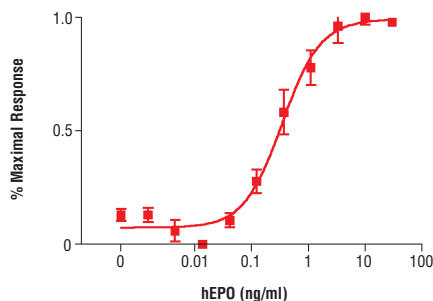
Endotoxin: Less than 0.01 ng endotoxin/1µg hEPO.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hEPO. All lots are greater than 98% pure.



The purity of recombinant hEPO was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hEPO and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hEPO was determined in a TF-1 cell proliferation assay. The ED₅₀ of each lot is between 50-400 pg/ml.



Background: EPO is a 34 kDa protein that is required for the survival and terminal differentiation of erythrocytes (1). EPO is produced by a number of cell types including tubular endothelial cells, interstitial cells, hepatocytes, Kupffer cells, astrocytes, and Schwann cells (2-4). EPO inhibits apoptosis and may protect neuronal cells from death during ischemia and/or neurodegenerative diseases (3,4). EPO inhibits the production of pro-inflammatory cytokines through inhibition of NF-κB signaling (5). EPO protected against the development of diabetes in an animal model by promoting pancreatic β cell survival and growth (6). Binding of EPO to its cognate receptor, EPOR, induces activation of Jak2, Stat5 and Akt (3-5).

Background References:

- (1) Wu, H. et al. (1995) *Cell* 83, 59-67.
- (2) Koury, S.T. et al. (1989) *Blood* 74, 645-51.
- (3) Keswani, S.C. et al. (2011) *Proc Natl Acad Sci USA*, 108, 4986-90.
- (4) Um, M. and Lodish, H.F. (2006) *J Biol Chem* 281, 5648-56.
- (5) Nairz, M. et al. (2011) *Immunity* 34, 61-74.
- (6) Choi, D. et al. (2010) *J Exp Med* 207, 2831-42.

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2 containing 20 µg BSA per 1 µg hEPO.

Carrier free: Lyophilized from a 0.22 µm filtered solution of PBS, pH 7.2.

Reconstitution:

With carrier: Add sterile PBS or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final hEPO concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS or PBS containing protein to minimize absorption of hEPO to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hEPO should be greater than 50 µg/ml.

Storage: Stable in lyophilized state at 4°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

◀ The proliferation of TF-1 cells treated with increasing concentrations of hEPO was assessed. After 48 hour treatment with hEPO, cells were incubated with a tetrazolium salt and the OD₄₅₀ - OD₆₅₀ was determined.