Human Phospho-Erythropoietin R (Y426) Antibody

Monoclonal Mouse IgG_1 Clone # 690710

Catalog Number: MAB6926

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Phospho-Erythropoietin R (Y426) in direct ELISAs and Western blots.
Source	Monoclonal Mouse IgG_1 Clone # 690710
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the human Erythropoietin R Y426 site Accession # P19235
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

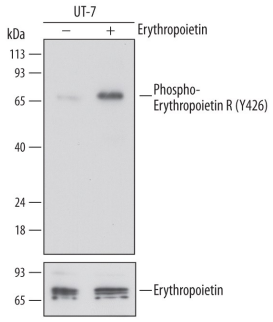
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Simple Western	10 µg/mL	See Below

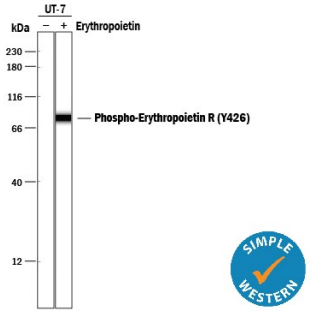
DATA

Western Blot



Detection of Human Phospho-Erythropoietin R (Y426) by Western Blot. Western blot shows lysates of UT-7 human acute myeloid leukemia cell line untreated (-) or treated (+) with 300 ng/mL Recombinant Human Erythropoietin (Tissue Culture Grade) (Catalog # 287-TC) for 10 minutes. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human Phospho-Erythropoietin R (Y426) Monoclonal Antibody (Catalog # MAB6926) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Phospho-Erythropoietin R (Y426) at approximately 64 to 70 kDa (as indicated). For additional reference, the membrane was stripped and reprobed with 1 µg/mL of Mouse Anti-Human Erythropoietin R Monoclonal Antibody (*lower panel*, Catalog # MAB3072). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western



Detection of Human Phospho-Erythropoietin R (Y426) by Simple Western™. Simple Western lane view shows lysates of UT-7 human acute myeloid leukemia cell line untreated (-) or treated (+) with 300 ng/mL Recombinant Human Erythropoietin (Tissue Culture Grade) (Catalog # 287-TC) for 10 minutes, loaded at 0.2 mg/mL. A specific band was detected for Phospho-Erythropoietin R (Y426) at approximately 70 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human Phospho-Erythropoietin R (Y426) Monoclonal Antibody (Catalog # MAB6926). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Erythropoietin (Epo), a glycoprotein produced primarily by the kidney, is the principal factor that regulates erythropoiesis by stimulating the proliferation and differentiation of erythroid progenitor cells. The biological effects of Epo are mediated by the erythropoietin receptor (Epo R). A member of the hematopoietic growth factor receptor superfamily which includes IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, G-CSF, Thrombopoietin, LIF, CNTF, Growth Hormone, and Prolactin, Epo R is expressed not only by erythroid cells but also by embryonic stem cells, endothelial cells, and neural cells (1). Mouse Epo R cDNA encode a type I membrane protein with 507 amino acid (aa) residues. Mouse Epo R has a 24 aa hydrophobic signal peptide, a 225 aa extracellular domain, a 22 aa transmembrane domain, and a 236 aa intracellular domain. At the protein sequence level, the human Epo R is approximately 82% identical to the mouse protein (2). Mouse and human Epo R both contain 11 cysteine residues and an N-linked glycosylation site. Mouse Epo R, however, contains two disulfide bridges not found with human Epo R. In common with other hematopoietic growth factor receptor superfamily members, mouse Epo R has 4 positionally conserved cysteines in its extracellular domain, a tryptophan-serine-X-tryptophan-serine (WSXWS) motif or its homolog located near the transmembrane region, and lacks kinase motifs in its intracellular domain. Based on its amino acid composition the molecular weight of Epo R would be 55 kDa but after post translational modification including glycosylation and tyrosine and serine-threonine phosphorylation the molecular weight can be as high as 78 kDa (1). As a result of alternative splicing of the Epo R mRNA, cDNA clones encoding a truncated form of the Epo R as well as a soluble form of Epo R have been found (2, 3). The presence of a soluble form of the Epo R has also been detected in human serum. Recombinant soluble Epo R binds Epo with high affinity and is a potent Epo antagonist (3).

References:

1. Spivak, J.L. (2001) in *Cytokine Reference*, Oppenhiem, J.J. and M. Feldmann, eds. Academic Press, New York, p. 941.
2. Kuramochi, S., Y. Ikawa and K. Todokoro (1990) J. Mol. Biol. **216**:567.
3. Baynes, R.D. *et al.* (1993) Blood **82**:2088.

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