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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Epo siRNA (r): sc-270111

BACKGROUND

Erythropoietin (Epo) is the primary factor responsible for regulating erythropoiesis during steady-state conditions and in response to blood loss and hemorrhage in the adult organism. In addition, Epo has been shown to play a role in primitive embryonic erythropoiesis. Epo is synthesized by the kidney and stimulates the proliferation and maturation of bone marrow erythroid precursor cells. Circulating Epo is a 165 amino acid glycoprotein. The Epo receptor, EpoR, is a glycoprotein expressed on megakaryocytes, erythroid progenitors and endothelial cells. Overexpression of Epo is associated with several pathophysiological conditions such as polycythemia vera, which is caused by the Epo-independent growth of erythrocytic progenitors from abnormal stem cells. A deficiency in Epo expression has been associated with afflictions such as anemia of chronic disease (ACD), frequently found in rheumatoid arthritis (RA) patients.

REFERENCES

1. Jelkmann, W. 1992. Erythropoietin: structure, control of production, and function. *Physiol. Rev.* 72: 449-489.
2. Dai, C.H., et al. 1992. Polycythemia vera. II. Hypersensitivity of bone marrow erythroid, granulocyte-macrophage, and megakaryocyte progenitor cells to interleukin-3 and granulocyte-macrophage colony-stimulating factor. *Blood* 80: 891-899.
3. Takahashi, T., et al. 1995. Characterization of three erythropoietin (Epo)-binding proteins in various human Epo-responsive cell lines and in cells transfected with human Epo-receptor cDNA. *Blood* 85: 106-114.
4. Lin, C.S., et al. 1996. Differential effects of an erythropoietin receptor gene disruption on primitive and definitive erythropoiesis. *Genes Dev.* 10: 154-164.

CHROMOSOMAL LOCATION

Genetic locus: Epo (rat) mapping to 12q12-q13.

PRODUCT

Epo siRNA (r) is a pool of 2 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Epo shRNA Plasmid (r): sc-270111-SH and Epo shRNA (r) Lentiviral Particles: sc-270111-V as alternate gene silencing products.

For independent verification of Epo (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270111A and sc-270111B.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Epo siRNA (r) is recommended for the inhibition of Epo expression in rat cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Epo (B-4): sc-5290 is recommended as a control antibody for monitoring of Epo gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Epo gene expression knockdown using RT-PCR Primer: Epo (r)-PR: sc-270111-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.