Erythropoietin (EPO)

ELISA Kit and Antibodies

Erythropoietin

The glycoprotein EPO, the main physiological regulator of red blood cell formation,¹ stimulates the proliferation of red blood cell progenitors and their differentiation into mature red blood cells. EPO is produced in the kidneys and its production is upregulated in response to low oxygen supply (hypoxia). This augments red blood cell production to meet increased demands due to blood loss, decreased ambient oxygen pressure or impaired oxygen supply to the tissues. EPO levels are altered in many diseases, including anemias, polycythemias, renal disorders, malignancies, infectious diseases, and inflammatory disorders.² Analysis of EPO levels in blood is important for research into the role of EPO in these diseases.

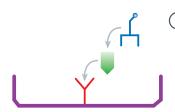
Importance Of Accurate Measurement

EPO levels in healthy persons are typically very low, on the order of 5 - 25 mU/mL (~2 - 10 pM). Accurate measurement of such low EPO levels and the distinction of normal from subnormal EPO levels (e.g. in patients with polycythemia vera) is important for detection. STEMCELL Technologies Inc.'s EPO ELISA Kit is an effective assay for detection of EPO because it has a strong signal-to-background ratio, as well as a detection limit well below normal EPO levels (<5 mU/mL).

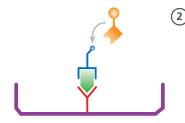
Why Use EPO Antibodies and ELISA Kit?

- SENSITIVE DETECTION. Two monoclonal antibodies are used in the ELISA to achieve a lower limit of detection of 0.6 mU/mL.
- ACCURATE RESULTS. The ELISA contains high affinity anti-EPO antibodies which do not cross-react with a panel of other human recombinant cytokines.
- REPRODUCIBLE DATA. Every batch of reagents used in the EPO ELISA Kit is extensively tested to ensure optimal performance and consistency.
- RAPID PROCEDURE. The ELISA is complete in 3 hours with an easy 3-step procedure (Figure 1).

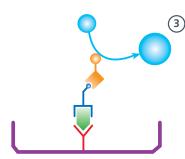
FIGURE 1. EPO ELISA 3-Step Procedure



Test samples and biotinylated anti-EPO detection antibody are incubated simultaneously in a 96-well microtiter plate that has been precoated with an anti-EPO capture monoclonal antibody.



 Wells are incubated with streptavidin conjugated to horseradish peroxidase (HRP).



3 TMB substrate solution is added to the wells and detects any HRP present. The reaction is stopped and the yellow product is measured spectrophotometrically. The color intensity is proportional to the amount of EPO present in each well.

- Y Anti-EPO monoclonal (capture) antibody immobilized in ELISA well
- EP
- Biotinylated anti-EPO monoclonal detection antibody
- Streptavidin-HRP conjugate
 - Tetramethylbenzidine (TMB) substrate reaction



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High Affinity Anti-EPO Reagents

For Consistent Results

EPO Antibodies

High affinity monoclonal antibodies against human EPO were generated by immunizing mice with pure human urinary EPO and purified from hybridoma culture by affinity chromatography. The antibodies, anti-human EPO 16 and anti-human EPO 26, recognize non-overlapping epitopes on EPO.

- Anti-Human EPO 16 used for: (1) immunoassay, (2) immunoaffinity isolation of human EPO, and
 (3) neutralization of human, mouse, and baboon EPO activity in a dose-dependent manner.³⁻⁶ This clone binds to a conformational epitope on EPO.
- Anti-Human EPO 26 used for: (1) immunoassay, (2) immunoaffinity isolation of human EPO, and
 (3) neutralization of human and baboon EPO in a dose-dependent manner.³⁻⁶ This clone binds to a linear epitope on EPO.

Ordering Information

PRODUCT	CLONE	ISOTYPE	QUANTITY	CATALOG #
Anti-Human EPO (anti- EPO 16)	16F1H11	Mouse IgG ₁	Purified 100 µg	01300
Anti-Human EPO (anti- EPO 26)	26G9C10	Mouse IgE	Purified 100 µg	01350

NOTE: The anti-EPO 16 and anti-EPO 26 antibodies are available in custom amounts Please contact us for more details.

Technical Information

Product Information Sheets, a Technical Manual, and a Mini Review about EPO are available at www.stemcell.com.

Comparison of Human EPO Antibodies

	EPO 16	EPO 26
Catalog #	01300	01350
Quantity	100 µg	100 µg
Isotype	Mouse IgG ₁	Mouse IgE
Format	Purified	Purified
Dissociation Constant	~8 nM	~0.7 nM
Cross Reactivity: Baboon EPO5 Mouse EPO5 Sheep EPO5 Horse EPO*	•	•

NOTE: When anti-EPO 16 and anti-EPO 26 are used together in a sandwich ELISA assay, the ELISA does not measure horse EPO in serum or plasma.

EPO ELISA Kit

The EPO ELISA Kit* is a rapid 3-step enzyme-linked immunosorbent assay (ELISA) designed for the quantitative measurement of natural and recombinant human EPO in biological fluids such as serum, plasma and tissue culture supernatants. The EPO ELISA Kit may also be used to detect recombinant human EPO or Darbepoetin (an EPO analog) in serum, plasma and urine of racehorses.

*For Research Use Only.

Characteristics of the EPO ELISA Kit

- SPECIFICITY: This assay utilizes two monospecific monoclonal antibodies raised against human urinary EPO that bind to non-overlapping epitopes on the EPO polypeptide and show high-affinity binding to both native and recombinant EPO³, and to the EPO analog Darbepoietin.
- SENSITIVITY: 0.6 mU/mL
- RANGE: Approximately 1.6 100 mU/mL
- ASSAY TIME: Approximately 3 hours
- TEST SAMPLE MATERIALS: Serum, plasma, culture supernatants, purified EPO preparations
- SAMPLE VOLUME NEEDED: 100 µL per duplicate assay

Ordering Information

PRODUCT	QUANTITY	CATALOG #
ELISA Kit for Human Erythropoietin	1 kit	01630

References

- Papayannopoulou T, et al., pp 242-254. In: Hematology
 Basic Principles and Practice. 2nd ed. New York: Churchill
 Livingstone Inc, 1995
- 2. Spivak JL, et al., Lancet 13: 1707-1712, 2000
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