For life science research only.
Not for use in diagnostic procedures.

EPO ELISA

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Photometric enzyme-linked immunosorbent assay (ELISA) for the quantitative *in vitro* determination of erythropoietin in human serum or plasma research samples using antibody-coated microplates.

Cat. No. 11 693 417 001 1 kit
96 tests
Not available in US

Store the kit at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	EPO ELISA, hEPO Control Serum	▪ Human serum ▪ Positive control ▪ Lyophilized ⚠ ***See lot-specific label for exact content in mIU/ml.***	1 bottle
2	blue	EPO ELISA, Anti-hEPO-POD (HRP)	▪ Monoclonal antibody to hEPO (from mouse, clone HE 1/5.5.11), conjugated to peroxidase. ▪ Detection antibody ▪ White lyophilizate	1 bottle
3a	sand	EPO ELISA, hEPO Standard	▪ Contains between 0 and approximately 200 mIU/ml EPO in a serum analogue matrix containing BSA, buffer, and preservatives. ⚠ ***See lot-specific label for exact content in mIU/ml*** ▪ White lyophilizate	1 bottle
3b	beige			1 bottle
3c	mustard			1 bottle
3d	olive			1 bottle
3e	caramel			1 bottle
3f	brown			1 bottle
4	white	EPO ELISA, Incubation buffer	▪ Ready-to-use solution. ▪ Clear solution with possible foaming.	1 bottle, 100 ml
5	green	EPO ELISA, Sample buffer	▪ Ready-to-use solution. ▪ Dilution buffer ▪ Clear or turbid yellow solution with possible foaming.	1 bottle, 100 ml
6	white	EPO ELISA, Washing buffer	White tablets	1 bottle, 2 tablets
7	black	EPO ELISA, TMB substrate solution	▪ Ready-to-use solution. ▪ Clear, slightly yellow solution.	1 bottle, 15 ml
8	colorless	EPO ELISA, TMB stop solution	▪ Ready-to-use clear solution. ▪ 0.94 N sulfuric acid	1 bottle, 7 ml
9	foil bag	EPO ELISA, Microplate	▪ Pre-coated with a polyclonal antibody to EPO (from rabbit). ▪ Shrink-wrapped with a desiccant capsule (12 × 8 wells).	1 strip frame, 12 modules of 8 wells each
10	–	EPO ELISA, Self-adhesive Plate Cover Foil	▪ Prevents evaporation. ⚠ ***Cover the Microplate modules with the Cover Foils during each incubation step.***	4 foils

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	green	hEPO Control Serum	Store at +2 to +8°C.
2	blue	Anti-hEPO-POD (HRP)	
3a	sand	hEPO Standard	
3b	beige		
3c	mustard		
3d	olive		
3e	caramel		
3f	brown		
4	white	Incubation buffer	
5	green	Sample buffer	
6	white	Washing buffer	
7	black	TMB substrate solution	
8	colorless	TMB stop solution	
9	foil bag	Microplate	
10	–	Self-adhesive Plate Cover Foil	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Microplate washer
- Microplate reader
- Microplate shaker
  If no shaker is used, signal levels will be considerably lower (approximately 40%) and may vary.
- Pipettes carefully calibrated
- Sterile aerosol-resistant pipette tips
- Sterile cups for preparing dilutions
- Absorbent, disposable towels for washing steps
  Use plastic disposables. Avoid glassware.

Data analysis

- ELISA reader
- Calculation software recommended

Adaptation to automation

The EPO ELISA can be used in automated microplate systems. The reagents are provided in excess (≥20%) for this purpose. With normal use, this is sufficient to perform the test in one cycle on most machines.

For the preparation of kit working solutions

- Double-distilled water

All reagents necessary to perform the assay are supplied with this kit.

1.4. Application

The EPO ELISA is designed for use in research studies as a method for the quantitative *in vitro* determination of natural and recombinant human EPO in serum and plasma within antibody-precoated microplates.

1.5. Preparation Time

Assay Time

The assay time is 3.5 hours.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The EPO ELISA can be used with the following sample materials:
- Serum
- Plasma, treated with EDTA, citrate, oxalate, or heparin.

⚠ ***Do not use samples which are clotted, grossly hemolyzed, lipemic, or microbially contaminated.***

⚠ ***Samples must not contain sodium azide or sulfide reducing agents, such as 2-mercaptoethanol and DTT because they will interfere with peroxidase activity of the detection antibody.***

General Considerations

Precautions

- Pipette thoroughly to ensure accurate transfer of the small volumes.
- Perform a separate calibration curve simultaneously with each test series.
- Perform all measurements in duplicates.
  ℹ *All reagents necessary to perform the assay are supplied with this kit.*
- Reagents and Microplate modules of different lots must not be used in one test series.
- Equilibrate all reagents to +15 to +25°C before use, except the TMB substrate which can be used directly from the refrigerator.
- The TMB substrate solution is very sensitive to contamination, therefore do not pipette directly from the bottle, instead transfer the required quantity into a separate vial.

Adaption for automation

The EPO ELISA can be used in automated microplate systems. The reagents are provided in excess (≥20%) for this purpose. With normal use, this is sufficient to perform the test in one cycle on most machines.

Number of tests

96 tests (48 duplicate determinations including a blank, six standards, and a control).

Safety Information

The blood of the donors for the control and standards was tested for the presence of HBsAg and antibodies to HIV, HIV-2, HCV, and found to be negative, according to the current quality control procedures. Since the danger of infection cannot be completely excluded, the positive control and standards must be handled with the same care as infected material. In the case of exposure, the guidelines of the appropriate health authorities must be followed.

The following reagents provided with the kit are toxic or corrosive and should be handled with care:
- TMB substrate solution (Bottle 7)
- TMB stop solution (Bottle 8)

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Content	Reconstitution/Preparation of Working Solution	Storage and Stability	For use in...
1	hEPO Control Serum (Bottle 1)	▪ Reconstitute the lyophilizate in 500 µl double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly. ▪ This results in a slightly milky solution. ⚠ ***Do not vortex.***	Store 1 week at +2 to +8°C or 12 months at −15 to −25°C.	Step 1
2	Anti-hEPO-POD (HRP) (Bottle 2)	▪ Reconstitute the lyophilizate in 500 µl double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly. ▪ This results in a clear, colorless solution. ⚠ ***Do not vortex.***	Store 12 months at +2 to +8°C. ⚠ ***Do not freeze.***	Solution 9
3a to 3f	hEPO Standard (Bottles 3a to 3f)	▪ Reconstitute the lyophilizate in 1 ml double-distilled water for 10 minutes at +15 to +25°C; mix thoroughly. ▪ This results in a clear, colorless solution. ⚠ ***Do not vortex.***	▪ Store 8 hours at +2 to +8°C. ▪ Store in aliquots at −15 to −25°C.	Step 1
4	Incubation buffer (Bottle 4)	▪ Ready-to-use clear solution.	Store at +2 to +8°C.	Solution 9
5	Sample buffer (Bottle 5)	▪ Ready-to-use solution. ▪ Clear or yellow solution.	Store at +2 to +8°C.	Step 1
6	Washing buffer (Bottle 6)	▪ Dissolve one tablet in 2 liters of double-distilled water. ▪ This results in a clear, colorless solution.	Store 12 months at +2 to +8°C.	Step 2
7	TMB substrate solution (Bottle 7)	▪ Ready-to-use solution. ▪ Clear or yellow solution.	Store at +2 to +8°C.	Step 3
8	TMB stop solution (Bottle 8)	▪ Ready-to-use clear solution.	Store at +2 to +8°C.	Step 4
9	Immunoreagent	For 100 wells (5 ml): ▪ Add 250 µl reconstituted Anti-hEPO-POD (Solution 2) to 4.75 ml Incubation buffer (Solution 4); mix thoroughly.	Store 8 hours at +2 to +8°C. ⚠ ***Prepare shortly before use.***	Step 1

⚠ ***All lyophilizates should become clear solutions after reconstitution except the positive control. Any particles within the reconstituted solution should be considered as deterioration. The immunoreagent, substrate solution, and washing buffer should be clear and colorless. Precipitates or cloudiness in the reagent solutions should be considered as indications of instability or deterioration.***

2.2. Protocols

Sample preparation serum

Perform the following steps when using serum.

1. Use a serum separator or clot tube and allow samples to coagulate for 30 minutes at +15 to +25°C.
   ⚠ ***Avoid hemolysis.***

2. Centrifuge the samples at 700 × g for 15 minutes at +15 to +25°C.

3. Collect the serum.

A minimum of 150 µl per assay (duplicate determination) should be collected.

– Aliquot samples and store at −15 to −25°C.

Sample preparation plasma

Perform the following steps when using plasma.

1. Collect plasma using EDTA, heparin, oxalate, or citrate as anticoagulant at +15 to +25°C.

2. Centrifuge the samples at 700 × g for 15 minutes at +15 to +25°C.

3. Collect the plasma.

A minimum of 150 µl per assay (duplicate determination) should be collected.

– Aliquot samples and store at −15 to −25°C.

Storage of samples

If the sample material cannot be used on the same day, aliquot and store samples at −15 to −25°C. For transport and shipping purposes, the following serum data has been collected.

Days of storage	Recovery (%) +4°C	Recovery (%) +25°C	Recovery (%) +37°C
0	100	100	100
1	86	86	87
4	90	88	54
7	82	83	60

Store sample material at +2 to +8°C for ≤4 days.

Predilution of samples and positive control

Serum, plasma, and the positive control can be used undiluted. Samples exceeding the measuring range should be diluted further with Sample buffer (Bottle 5) and remeasured. Maximum dilution in Sample buffer should not exceed 1:10. The dilution factor should be taken into account when calculating the results.

Microplate pipetting scheme

Set up the pipetting scheme according to the following table.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bl	Bl	P1	P1	–	–	–	–	–	–	–	–
B	Sa	Sa	–	–	–	–	–	–	–	–	–	–
C	Sb	Sb	–	–	–	–	–	–	–	–	–	-
D	Sc	Sc	–	–	–	–	–	–	–	–	–	–
E	Sd	Sd	–	–	–	–	–	–	–	–	–	–
F	Se	Se	–	–	–	–	–	–	–	–	–	–
G	Sf	Sf	–	–	–	–	–	–	–	–	–	–
H	P0	P0	–	–	–	–	–	–	–	–	P40	P40

Bl = blank (only substrate and stop solution)
Sa to Sf = hEPO standards
P0 = positive control (hEPO Control Serum)
P1 to P40 = samples 1 to 40
Perform a separate calibration curve simultaneously with each test series.
Perform all measurements in duplicates.

ELISA assay

Follow these steps to determine the amount of EPO in serum or plasma samples.

⚠ ***Equilibrate reagents to +15 to + 25°C before starting the assay. TMB substrate solution (Bottle 7) can be used directly from the refrigerator. Reagents from kits with different lot numbers must not be used in one assay series.***

Perform all incubation steps at +15 to +25°C. Always use the same procedure to minimize inter-assay variances.

1. Pipette 30 µl of the Sample buffer (Bottle 5) into all the wells used for the EPO Standards, the positive control, and the sample material.
   – Add into the appropriate wells, 20 µl of EPO Standards (Solutions 3a to 3f), or the positive control (Solution 1), or the sample material (serum or plasma).

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2. Add 50 µl of the Immunoreagent (Solution 9).
   – Cover the Microplate modules with the Cover Foil and incubate for 3 hours under constant shaking at 300 rpm.

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3. Remove the solution by aspirating away the buffer.
   Alternatively, the Microplate may be inverted and tapped gently on a paper towel.
   – Rinse wells 3 times with 300 µl of Washing buffer (Solution 6) for 1 minute each.

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4. Carefully remove Washing buffer by aspirating or tapping.
   – Pipette 100 µl of TMB substrate solution (Bottle 7) into each well.

⚠ ***This reagent is very sensitive to contamination. Do not pipette directly from the TMB substrate bottle (Bottle 7); transfer the required quantity into a separate tube.***

– Incubate at +15 to +25°C until color development is sufficient for photometric detection, approximately ≤20 minutes.
– Shake Microplates at 300 rpm during incubation with substrate solution.

Measurement

Measurements can be done with or without TMB stop solution.

① Without TMB stop solution (Bottle 8): measure the absorbance of the samples in an ELISA reader at 370 nm (reference wavelength approximately 492 nm).

ℹ *Not stopping the substrate reaction allows repeated measurement at various times points, for example, every 2 minutes and to follow the kinetics of color development.*

② With stop solution (Bottle 8): add 25 µl of TMB stop solution to each well and incubate the microplate for approximately 1 minute on a shaker at 300 rpm.
– Measure within 5 minutes the absorbance of the samples in an ELISA reader at 450 nm (reference wavelength 690 nm).

ℹ *Color will begin to fade after 5 minutes.*
ℹ *Measurement at 450 nm will result in a two to threefold increase of absorbance values, but does not allow kinetic of color development to be followed.*

2.3. Parameters

Accuracy

Characteristic	Data
Calibration	The standards and the control are calibrated against the 2nd International WHO Reference Preparation 67/343 (natural human EPO).
Dilution test	Target ± 20% Definition: Three sera were spiked with three different EPO concentrations and then diluted in 1:2 dilution steps with either serum or sample buffer. The resulting EPO concentration was then determined in triplicates. The average determined recovery in serum was >80%, up to a dilution of 1:16 and in sample buffer, up to a dilution of 1:8.
Recovery	EDTA plasma >80%
	Serum 100%
	Definition: For the evaluation of the recovery, one serum or plasma was spiked with a high concentration of EPO and either diluted in log 2 dilution steps with a serum of a known low concentration of EPO or in sample buffer, respectively. The concentration in all samples was determined in fourfold measurements. The average determined recovery was 99.5% (+/-6) up to a dilution of 1:32 in serum, or in sample buffer of 93% (+/-3) up to a dilution of 1:8, respectively.
High dosage hook effect	No high dosage hook effect has been observed in serum and plasma spiked with up to 4,000 mIU/ml EPO. This concentration is 20 times higher than the highest standard supplied with the kit.
Interferences	Human anti-mouse antibodies: no interference
	Bilirubin (up to 384 µmol/l): no influence
	Lipid (up to 810 mg/dl): no influence
	Hemoglobin (>40 mg/dl to 400 mg/dl): 1.5 times higher EPO concentration
	Aspirin (up to 300 µg/ml): slightly increased EPO concentration
	Phenytoin (up to 20 µg/ml): slightly increased EPO concentration

Detection range

Approximately 3 to 200 mIU/ml; conversion factor: 1 mIU/ml = 6.6 pg/ml.
Definition: The measuring range is between the limit of quantification (2.8 mIU /ml), and the highest standard (Bottle 3f, approximately 200 mIU EPO/ml).

Precision

Characteristic	Data
Sensitivity, Limit of Detection (LOD)	≥0.24 mIU/ml Definition: The LOD is the lowest concentration of an analyte that the analytical process can reliably differentiate from background levels. It is defined as the absorbance mean of 21 replicates of the zero standard plus threefold the standard deviation reading from the standard curve.
Sensitivity, Limit of Quantification (LOQ)	≥2.8 mIU/ml Definition: The LOQ is the lowest concentration of an analyte that can be measured with a stated level of confidence. It is defined as the minimum detectable concentration of EPO in 5 replicates showing a coefficient of variation <15% and where the 3 SD range does not overlap to 3 SD of the 0-standard. This value is higher than LOD.
Intra-assay variance	7.1% (low sample) 2.7% (medium sample) 3.9% (high sample) Definition: To determine the intra-assay variance, three different concentrations (low, medium, and high) of EPO were added to one serum. The resulting EPO concentrations were then determined in twentyfold measurement with three different lots of kits.
Inter-assay variance	8.3% (low sample) 1.9% (medium sample) 2.8% (high sample) Definition: To determine the inter-assay variance, three different concentrations of EPO (low, medium, and high) were added to one serum. The resulting EPO concentrations were then determined in twentyfold measurements on ten successive days. A variance of 7.2% was established.
Linearity	In serum: r ≥0.99 In sample buffer: r ≥0.99

Specificity

The ELISA system detects both natural and recombinant human EPO from CHO cells. No cross-reaction with other serum components has been found.

3. Results

Plotting the standard curve

Use the standards provided in the kit to prepare a six point calibration curve.

1. Correct each absorbance value of all standards by subtracting the value of the reagent blank (Bl = only substrate and stop solution).

2. Calculate the mean absorbance value for each standard from the duplicates.

3. Prepare a plot correlating the mean absorbance values of the standards to the analyte concentrations of the standards.
   The lot-specific concentration of each standard is listed on its bottle label.

4. Prepare the plot using either of two methods:

Method	Steps
Automatic	Enter the absorbance values and the analyte concentrations into a suitable data analysis software program. *To achieve best results, make sure the software is able to calculate the standard curve with a four-parameter Rodbard-function. Several data analysis programs perform statistical analysis, such as recalculation, mean, SD, CV, CV regression analysis on the absorbance values entered.*
Manual	Plot on semilogarithmic graph paper, the mean absorbance values on the Y-axis against the analyte concentrations on the X-axis.

Determination of the analyte concentration

Follow these steps to determine the analyte concentration:

1. Correct each absorbance value of all samples and controls by subtracting the value of the reagent blank (Bl= only substrate and stop solution).

2. Calculate the mean absorbance value for each sample from the values of the duplicates.
   If the absorbance of the sample duplicates are above the absorbance of the highest standard, do not use those duplicates to determine analyte concentration in the diluted sample (next step). Repeat the assay for that sample as outlined in the next section, **Handling very concentrated samples**.

3. Determine the analyte concentration in the diluted sample using either of two methods:

Method	Steps
Automatic	Enter the mean of the sample absorbance values into a suitable data analysis software program to prepare the standard curve, see section, **Plotting the standard curve**. *The data analyte program will automatically determine the analyte concentration by comparing the absorbance of the sample to the standard curve.*
Manual	Locate the mean sample absorbance on the Y-axis of the standard curve (**Step 4, Plotting the standard curve**) and read from the X-axis, the analyte concentration that corresponds to the specific absorbance value.

Handling very concentrated samples

Dilute samples exceeding the measuring range with Sample buffer (Bottle 5) and repeat the ELISA. Maximum dilution in Sample buffer (Bottle 5) should not exceed 1:20. This dilution factor must be considered when calculating the content of EPO.

Typical results

The standard curve must be determined individually for each experiment. An example is shown in Figure 1.

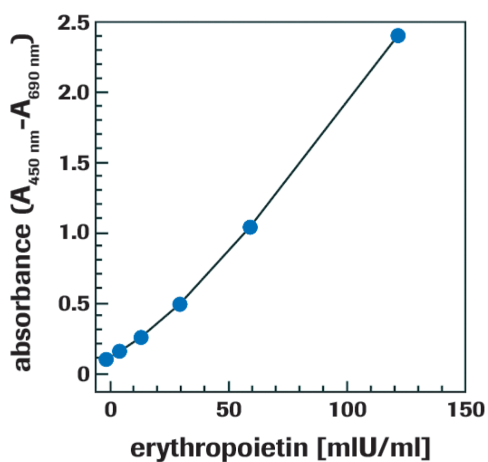

Fig. 1: Typical standard curve.

4. Troubleshooting

Observation	Possible cause	Recommendation
Unexpected color development.	Inadequate incubation time and temperature.	Ensure that incubation intervals are correct and that all reagents equilibrate to +15 to +25°C prior to use.
	Poor quality water negatively influences the test.	Always use double-distilled water for reconstitution and preparing the working solutions. ⚠ ***Water must not be microbially contaminated.***
	Substrate or vial used to aliquot substrate contaminated with oxidative active substances.	Do not pipette directly from the substrate bottle.
		Check the vial for contamination.
Questionable readings obtained.	Nonsuitable filters in the Microplate reader have been used.	Check the filters in your Microplate reader for the correct wavelength.
Weak or no signal present.	Sodium azide, 2-mercaptoethanol, and DTT interfere with the peroxidase activity.	Only use samples and solutions without sodium azide, 2-mercaptoethanol, or DTT.
Drift	Unequal distribution of temperature in the wells.	Ensure that all reagents equilibrate to +15 to +25°C prior to the assay, except TMB substrate solution which can be used directly from the refrigerator.
		Use the recommended incubation times and temperatures.
	Evaporation of fluids.	Check for adequate placement of the adhesive cover foils during the incubation steps.
Poor precision	Non-homogeneous sample after freezing.	Mix sample completely before pipetting.
	Turbidity, particles, or high lipid content of the sample.	Centrifuge sample to pellet particles.
		Mix sample well before pipetting.
	Carryover between samples and standards.	Change pipette tips between each pipetting step.
	Unequal volumes added to the wells.	Check pipette function, and recalibrate if necessary.
	Inadequate aspiration of fluids.	Aspirate completely; no fluid should remain in the wells after aspiration.
	Washing was incomplete.	Ensure that the automatic washer is working properly.
	Unequal mixing of reagents during incubation.	Use a plate shaker to ensure adequate mixing.

5. Additional Information on this Product

5.1. Test Principle

Test principle

The EPO ELISA is based on the sandwich ELISA principle. Antibodies to EPO (anti-EPO) are prebound to the surface of the Microplate modules (Fig. 2).

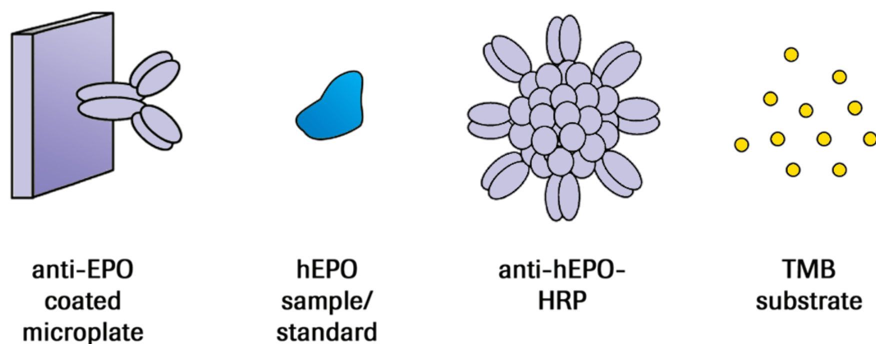

Fig. 2: Test principle

1. EPO is simultaneously bound to the anti-EPO-coated surface of the microplate and to the peroxidase-conjugated detection antibody.

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2. Following the washing step, the peroxidase (POD) bound in the complex is developed by the substrate tetramethylbenzidine (TMB), and determined photometrically.
   – The color intensity is proportional to the concentration of EPO.

Preparation

EPO is produced by:
- Specialized renal cells.
- Regenerating human hepatic cells.

Background information

Genetic properties

The human gene for EPO is a single-copy gene and localized on chromosome 7.

Molecular properties

EPO is an acidic glycoprotein.
- The immature protein consists of 193 amino acids. A 27 amino acid leader from the N-terminus, and a single arginine residue from the C-terminus are cleaved to form the mature EPO polypeptide.
- The mature protein consists of 165 amino acids.

Molecular weight

Due to variations of the carbohydrate side chains which are highly variable, the molecular weight might vary between 30 and 34 kDa.

Protein structure

- EPO contains two disulfide bonds, at least one of which is critical for the biological activity of the molecule.
- The single polypeptide chain is responsible for stimulating the target cells.
- The four carbohydrate side chains determine the stability and pharmacokinetic characteristics of the molecule, especially the rate at which EPO is eliminated from the bloodstream.

Regulation

EPO production is regulated by:
- Hormones, such as renin, angiotensin II, or epinephrine.
- Cytokines, such as IL-1, IL-6, or TNF.
- Amount of oxygen supply to the site of EPO production.

Biological function

EPO is the only growth factor with a hormone-like effect:
- Acts on precursors of red blood cells (erythrocytes) in the bone marrow.
- Enhances the proliferation and differentiation of these precursor cells into erythrocytes.

Molecule in disease

In certain forms of anemia, especially in aplastic renal anemia, the regulation of EPO formation in the kidney appears to be intact. This leads to an extremely high EPO concentration in serum of the respective individuals, up to 4 U/ml. Other forms of anemia, especially renal anemia, are characterized by serum EPO concentrations lower than expected with respect to the degree of anemia. In these subjects, there is obviously a disorder in the regulation of EPO formation.
Further forms of anemia are known where it is not established whether they are based on an EPO deficiency. This question may be assessed by determining the EPO concentration in serum of respective individuals and putting the results into relation to the hemoglobin concentration (or the hematocrit value). Exaggerated EPO levels accompanied by a normal or even elevated hemoglobin concentration (and/or hematocrit values) have been found in a series of diseases, such as tumors of the kidney or liver, or in secondary forms of polycythemia. Polycythemia vera, however, is characterized by an abnormally increased red blood cell count and a concomitantly low EPO serum level.
The EPO ELISA is intended to help increase the scientific knowledge about these relationships.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Trademarks

All product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications,
please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

1220.11740792001 ⑩

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