ab119522

Erythropoietin (EPO) Human ELISA Kit (with color giving dyes)

Instructions for Use

For the quantitative measurement of Human Erythropoietin (EPO) concentrations in cell culture supernatant, serum and plasma (EDTA, citrate, heparin)

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 2 September 2013

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1. Introduction

Erythropoietin (EPO) is a hormone produced by the kidney that promotes the formation of red blood cells in the bone marrow. EPO is a glycoprotein with a molecular weight of 34,000.

The kidney cells that make EPO are specialized and sensitive to low oxygen levels in the blood. These cells release EPO when the oxygen level is low in the kidney. EPO then stimulates the bone marrow to produce more red cells and thereby increase the oxygen-carrying capacity of the blood.

EPO is the prime regulator of red blood cell production. Its major functions are to promote the differentiation and development of red blood cells and to initiate the production of hemoglobin, the molecule within red cells that transports oxygen.

The EPO gene has been found on Human chromosome 7 (in band 7q21). EPO is produced not only in the kidney but also, to a lesser extent, in the liver. Different DNA sequences flanking the EPO gene act to control kidney versus liver production of EPO.

The measurement of EPO in the blood is useful in the study of bone marrow disorders and kidney disease. Elevated levels of EPO can be seen in polycythemia, a disorder in which there is an excess of red blood cells. Lower than normal levels of EPO are seen in chronic

renal failure. EPO plays an important role in the brain's response to neuronal injury. EPO is also involved in the wound healing process.

Measurement of serum immunoreactive erythropoietin has shown that overproduction of EPO can be an adaptive response to conditions producing tissue hypoxia, such as smoking chronic obstructive pulmonary disease, renal hypoxia or cyanotic heart disease. Elevated serum levels have further been found in patients suffering from various neoplastic diseases, such as renal carcinomas and benign renal tumors, liver carcinomas and hepatomas, cerebellar hemangioblastomas and other neoplastic disorders.

Serum levels of EPO lower than normal are found in various forms of anemias. Proinflammatory cytokines hereby act in lowering erythropoietin production. Mainly IL-1 and TNF-alpha seem to be responsible for the defect in EPO production in severe systemic and renal inflammatory diseases. These include anemia of renal failure, anemias of chronic disorders like in AIDS or rheumatoid arthritis, anemias due to hypothyroidism, malnutrition, and prematurity.

In case EPO is not primarily involved in the cause of the anemia, elevated levels of EPO are found in serum of the patients suffering from diseases like aplastic anemias, iron deficiency, megaloblastic anemias, thalassemias and myelodysplastic syndromes.

2. Assay Summary

An anti-Human Erythropoietin coating antibody is adsorbed onto microwells.

Human Erythropoietin present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-Human Erythropoietin antibody is added and binds to Human Erythropoietin captured by the first antibody.

Following incubation unbound biotin conjugated anti-Human Erythropoietin antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-Human Erythropoietin antibody.

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A colored product is formed in proportion to the amount of Human Erythropoietin present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 Human Erythropoietin standard dilutions and Human Erythropoietin sample concentration determined.

3. Kit Contents

Item	Quantity
Aluminium pouch with a Microwell Plate coated with monoclonal antibody to Human Erythropoietin (EPO)	1
Biotin-Conjugate anti-Human Erythropoietin (EPO) monoclonal antibody	70 µl
Streptavidin-HRP	150 µl
Human Erythropoietin Standard lyophilized (200 mIU/ml upon reconstitution)	2 vials
High control, lyophilized	1 vial
Low control, lyophilized	1 vial
Sample Diluent	12 ml
Assay Buffer Concentrate (20x) (PBS with 1% Tween 20 and 10% BSA)	5 ml
Wash Buffer Concentrate (20x) (PBS with 1% Tween 20)	50 ml
Substrate Solution (tetramethyl-benzidine)	15 ml
Stop Solution (1M Phosphoric acid)	15 ml

Item	Quantity
Blue-Dye	0.4 ml
Green-Dye	0.4 ml
Red-Dye	0.4 ml
Adhesive Films	4

4. Storage and Handling

- Store kit reagents between 2° and 8°C except controls. Store lyophilized controls at -20°C.
- Immediately after use remaining reagents should be returned to cold storage (2° to 8°C).
- Expiry of the kit and reagents is stated on labels. Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

5. Additional Materials Required

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips.
- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips.
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system).
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length).
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

6. Specimen Collection and Storage

Cell culture supernatant, serum and plasma (EDTA, citrate, heparin), were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible “Hook Effect” due to high sample concentrations.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive Human Erythropoietin. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7. Preparation of Reagents

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

A. Wash Buffer (1x)

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 – 12	50	950

B. Assay Buffer (1x)

Pour the entire contents (5 ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 – 6	2.5	47.5
1 – 12	5.0	95.0

C. Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (1x) (ml)
1 – 6	0.03	2.97
1 – 12	0.06	5.94

D. Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 – 6	0.06	5.94
1 – 12	0.12	11.88

E. Human Erythropoietin Standard

1. Reconstitute **Human Erythropoietin standard** by addition of distilled water.

2. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 200.0 mIU/ml). Allow the reconstituted standard to sit for 10-30 minutes. Mix well prior to making dilutions.

3. After usage remaining standard cannot be stored and has to be discarded.

4. **Standard dilutions** can be prepared directly on the microwell plate or alternatively in tubes.

F. External Standard Dilution

1. Label 7 tubes, one for each standard point.
   S1, S2, S3, S4, S5, S6, S7

2. Then prepare 1:2 serial dilutions for the standard curve as follows:

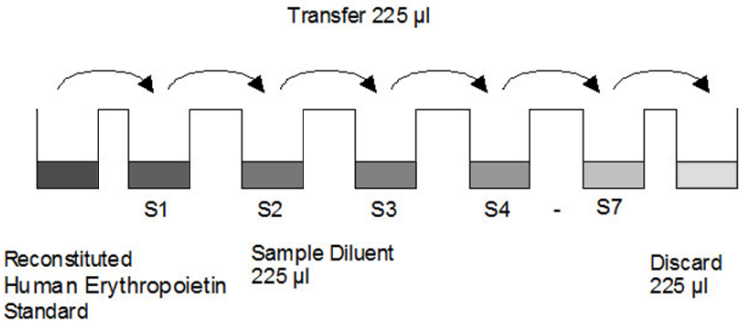
   - Pipette 225 µl of Sample Diluent into each tube.

- Pipette 225 µl of reconstituted standard (concentration of standard = 200.0 mIU/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 100.0 mIU/ml).

- Pipette 225 µl of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.

- Repeat serial dilutions 5 more times thus creating the points of the standard curve.

- Sample Diluent serves as blank.



G. Controls

Reconstitute by adding 250 µl distilled water to lyophilized controls (10-30 minutes). Swirl or mix gently to ensure complete and homogeneous solubilization. Further treat the controls like your samples in the assay. For control range please refer to certificate of analysis or vial label. Store reconstituted controls aliquoted at -20°C. Avoid repeated freeze and thaw cycles

H. Addition of Color-giving Reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help our customers to avoid any mistakes in pipetting the ELISAs, Abcam offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the protocol booklet.

Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye, Red-Dye***) can be added to the reagents according to the following guidelines:

1. **Diluent:** Before standard and sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the protocol booklet.

5 ml Sample Diluent	20 µl ***Blue-Dye***
12 ml Sample Diluent	48 µl ***Blue-Dye***
50 ml Sample Diluent	200 µl ***Blue-Dye***

2. **Biotin-Conjugate:** Before dilution of the concentrated Biotin-Conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the protocol booklet: Preparation of Biotin-Conjugate.

3 ml Assay Buffer (1x)	30 µl ***Green-Dye***
6 ml Assay Buffer (1x)	60 µl ***Green-Dye***
12 ml Assay Buffer (1x)	120 µl ***Green-Dye***

3. **Streptavidin-HRP:** Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the protocol booklet: Preparation of Streptavidin-HRP.

6 ml Assay Buffer (1x)	24 µl ***Red-Dye***
12 ml Assay Buffer (1x)	48 µl ***Red-Dye***

8. Assay Method

8.1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2° to 8°C sealed tightly.

8.2. Wash the microwell strips twice with approximately 400 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

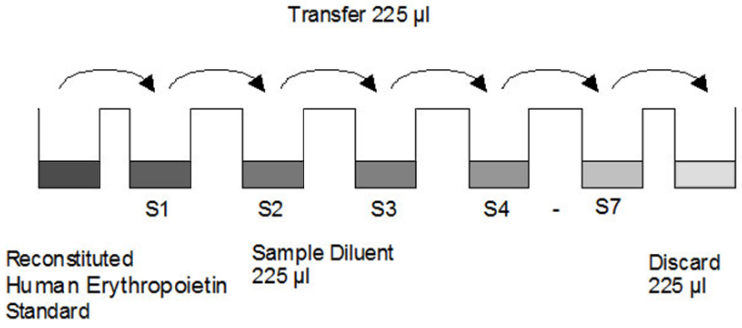
8.3. After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.

8.4. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes):

- Add 100 µl of Sample Diluent in duplicate to all **standard wells**.

- Pipette 100 µl of prepared **standard** (concentration = 200.0 mIU/ml) in duplicate into well A1 and A2 (see Table 1).

- Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 100 mIU/ml), and transfer 100 µl to wells B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of Human Erythropoietin standard dilutions ranging from 100.0 to 1.6 mIU/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.



In case of an **external standard dilution**, pipette 100 µl of these standard dilutions (S1 - S7) in the standard wells according to Table 1.

Table 1. Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (100.0 mIU/ml)	Standard 1 (100.0 mIU/ml)	Sample 1	Sample 1
B	Standard 2 (50.0 mIU/ml)	Standard 2 (50.0 mIU/ml)	Sample 2	Sample 2
C	Standard 3 (25.0 mIU/ml)	Standard 3 (25.0 mIU/ml)	Sample 3	Sample 3
D	Standard 4 (12.5 mIU/ml)	Standard 4 (12.5 mIU/ml)	Sample 4	Sample 4
E	Standard 5 (6.3 mIU/ml)	Standard 5 (6.3 mIU/ml)	Sample 5	Sample 5
F	Standard 6 (3.1 mIU/ml)	Standard 6 (3.1 mIU/ml)	Sample 6	Sample 6
G	Standard 7 (1.6 mIU/ml)	Standard 7 (1.6 mIU/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

8.5. Add 100 µl of Sample Diluent in duplicate to the blank wells.

8.6. Add 50 µl of Sample Diluent to the sample wells.

8.7. Add 50 µl of each sample in duplicate to the sample wells.

8.8. Prepare Biotin-Conjugate.

8.9. Add 50 µl of Biotin-Conjugate to all wells.

8.10. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 3 hours, if available on a microplate shaker set at 100 rpm.

8.11. Prepare Streptavidin-HRP.

8.12. Remove adhesive film and empty wells. Wash microwell strips 6 times according to step 2 of the test protocol. Proceed immediately to the next step.

8.13. Add 100 µl of diluted Streptavidin-HRP to all wells, including the blank wells.

8.14. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour, if available on a microplate shaker set at 100 rpm.

8.15. Remove adhesive film and empty wells. Wash microwell strips 6 times according to step 2 of the test protocol. Proceed immediately to the next step.

8.16. Pipette 100 µl of TMB Substrate Solution to all wells.

8.17. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

8.18. Stop the enzyme reaction by quickly pipetting 100 µl of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the

microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

8.19. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

9. Data Analysis

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the Human Erythropoietin concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating Human Erythropoietin for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Human Erythropoietin concentration.

- **If instructions in this protocol have been followed samples have been diluted 1:2 (50 µl sample + 50 µl Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**

- **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low Human Erythropoietin levels (Hook Effect). Such samples require further external pre-dilution according to expected Human Erythropoietin values with Sample Diluent in order to precisely quantitate the actual Human Erythropoietin level.**

- It is suggested that each testing facility establishes a control sample of known Human Erythropoietin concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in section A, Typical Data. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

A. Typical Data

Representative standard curve for Human Erythropoietin ELISA. Human Erythropoietin was diluted in serial 2-fold steps in Sample Diluent. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

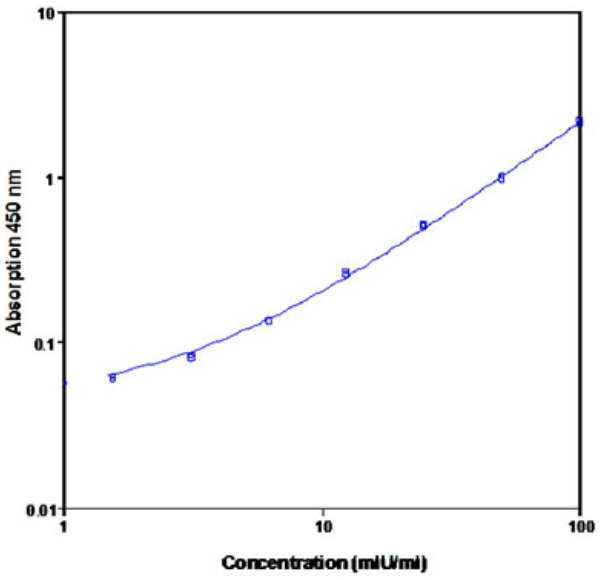


Table 2. Typical data using the Human Erythropoietin ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human Erythropoietin Concentration (mIU/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	100.0	2.189 2.083	2.136	2.5
2	50.0	1.012 0.965	0.988	2.4
3	25.0	0.499 0.514	0.507	1.4
4	12.5	0.256 0.268	0.262	2.2
5	6.3	0.134 0.135	0.134	0.2
6	3.1	0.080 0.083	0.081	1.7
7	1.6	0.062 0.059	0.061	1.8
Blank	0	0.023 0.026	0.024	6.0

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

B. Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

- The use of radioimmunotherapy has significantly increased the number of patients with Human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins

(serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

C. Sensitivity

The limit of detection of Human Erythropoietin defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.4 mIU/ml (mean of 6 independent assays).

D. Reproducibility

Intra-Assay: Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of Human Erythropoietin. 2 standard curves were run on each plate. Data below show the mean Human Erythropoietin concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 7.8%.

Table 3. The mean Human Erythropoietin concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human Erythropoietin Concentration (mIU/ml)	Coefficient of Variation (%)
1	1	7	7.6
	2	7	7.8
	3	6	10.8
2	1	60	3.8
	2	63	2.7
	3	57	4.1
3	1	7	5.7
	2	6	10.9
	3	6	7.6
4	1	15	9.2
	2	16	11.5
	3	13	4.3
5	1	16	12.1
	2	17	8.9
	3	13	8.7
6	1	15	4.7
	2	14	10.2
	3	14	9.5

Inter-Assay: Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 4 replicates of 6 serum samples containing different concentrations of Human Erythropoietin. 2 standard curves were run on each plate. Data below show the mean Human Erythropoietin concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 8.5%.

Table 4. The mean Human Erythropoietin concentration and the coefficient of variation of each sample

Sample	Mean Human Erythropoietin Concentration (mIU/ml)	Coefficient of Variation (%)
1	7	10.6
2	60	5.6
3	6	11.7
4	14	8.2
5	15	12.9
6	14	1.9

E. Recovery

The spike recovery was evaluated by spiking 4 levels of Human Erythropoietin into pooled normal serum samples. Recoveries were determined in 3 independent experiments with 4 replicates each. The amount of endogenous Human Erythropoietin in unspiked serum was subtracted from the spike values. The recovery ranged from 71% to 93% with an overall mean recovery of 81%.

F. Dilution Parallelism

Serum samples with different levels of Human Erythropoietin were analysed at serial 2 fold dilutions with 4 replicates each. The recovery ranged from 97.4% to 119.2% with an overall recovery of 105.2% (see Table 5).

Table 5.

Sample	Dilution	Expected Human Erythropoietin Concentration (mIU/ml)	Observed Human Erythropoietin Concentration (mIU/ml)	Recovery of Expected Human Erythropoietin Concentration (%)
1	1:2		70	
	1:4	35	35	101.9
	1:8	17	18	105.0
	1.16	9	10	116.1
2	1:2		52	
	1:4	26	25	97.4
	1:8	13	13	97.6
	1.16	7	7	104.4
3	1:2		68	
	1:4	34	34	99.9
	1:8	17	18	102.8
	1.16	9	9	106.9
4	1:2		59	
	1:4	30	30	102.3
	1:8	15	16	109.5
	1.16	7	9	119.2

10. Specificity

The assay detects both natural and recombinant Human Erythropoietin.

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into serum. There was no crossreactivity detected.

11. Expected Values

Panels of 40 serum as well as EDTA, citrate and heparin plasma samples were tested for Human Erythropoietin. Levels measured may vary with the sample collection used.

For detected Human Erythropoietin levels see Table 6.

Table 6.

Sample Matrix	Number of Samples Evaluated	Range (mIU/ml)	Mean of Detectable (mIU/ml)	Standard Deviation (mIU/ml)
Serum	40	2.7 – 40.7	12.9	9.6
Plasma (EDTA	40	1.6 – 57.2	8.5	10.3
Plasma (Citrate)	40	0.9 – 28.6	4.7	5.1
Plasma (Heparin)	40	2.1 – 41.9	13.7	9.0

12. Sample Stability

A. Freeze-Thaw Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 3 times, and the Human Erythropoietin levels determined. There was no significant loss of Human Erythropoietin immunoreactivity detected by freezing and thawing.

B. Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the Human Erythropoietin level determined after 24 h. There was no significant loss of Human Erythropoietin immunoreactivity detected during storage under above conditions.

For technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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