EPO Semi Quantification Kit

Directions for Use 101490/05

Issued: Aug 2019, Revised: June 2020

INTENDED USE

EPO Semi Quantification Kit is a lateral flow immunoassay for semi quantification of recombinant human erythropoietins, erythropoietin analogues or endogenous erythropoietin in EDTA-Plasma, serum or buffered samples. It is intended for research use only.

SUMMARY AND EXPLANATION

Erythropoietin (EPO) is a glycoprotein hormone of approximately 30 kDa whose main function is control of haematopoiesis. In adults, the majority of EPO is produced in the kidney. After posttranslational modifications hEPO has a characteristic isoform pattern different from most rhEPOs or EPO analogues. These patterns can be detected with techniques such as isoelectric focusing (IEF), SARCOSYL polyacrylamide gel electrophoresis (SAR-PAGE) and Membrane Assisted Isoform ImmunoAssay (MAIIA). Since EPO concentration in biological fluids is highly variable, the EPO semi quantification kit is designed to estimate sample to sample variability by measuring the total EPO concentration. This allows for multitesting of samples to be performed in a more efficient and uniform level.

PRINCIPLE OF THE PROCEDURE

An immobilised anti-EPO antibody is used to bind EPO in a thin detection zone. The EPO bound is visualized with Anti-EPO Carbon Black Nano-Strings (Anti-EPO CBNS) in a sandwich configuration yielding a black to grey signal. The intensity is proportional to the amount of bound EPO in the detection zone. The concentration of EPO in the sample can roughly be estimated visually by comparing the signal from standards with known rhEPO concentration.

REAGENTS

Art No	Name and Contents		
1450	EPO Semi Quantification Kit		
	Contains reagents for 100 tests.		
	Contents:		
	1x EPO total strips (red), 100 pcs	Ready for use	101002
	1x Sample buffer, 15mL [(a)*]	Ready for use	101520
	1x Anti-EPO CBNS, 3 mL [(a)]	Stock solution	100064
	1x CBNS dilution buffer, 3 mL [(a)]	Stock solution	100075
	1x Washing buffer, 3 mL [(a)]	Ready for use	100080
	1x Microplate, 5 pcs	Ready for use	101530
	1x Strip holder, 3 pcs	Ready for use	101211
	1x Scanning template, 6 pcs	Ready for use	100192

[(a)] Contains <0.1% sodium azid

Storage and Shelf Life

Store all components at +4-8°C. Do not freeze kit components. For expiration dates, see the product label.

Precautions

- Not for internal or external use in humans or animals.
- Not for *in vitro* diagnostic use.
- Do not use reagents beyond their expiration dates.
- Contamination of reagents may yield incorrect results.
- Always use good laboratory procedures when handling the product and wear suitable protective clothing.
- Human body fluids must be handled and treated as potentially infectious.
- Do not substitute kit reagents with those from other lots or other sources.

Warning! Products that contain sodium azide as a preservative must be handled with care. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by Centers of Disease Control and Prevention (CDC) or other local/national guidelines.

SAMPLE PREPARATION

EPO references

Prepare EPO references: 0, 10, 20, 50, 100 and 200 ng/L Epoetin (α/β) in Sample buffer as recommended. 100 µL is needed per sample. Store the EPO references according to manufacturer's recommendation.

Note! Different EPO variants affect the signal intensity in the strips diffrently. Chose EPO references that closely relates to your samples.

EDTA-Plasma or Serum Samples

Transfer 30 µL EDTA-plasma or serum and 120 µL sample buffer into a microtube. Vortex gently and let incubate for 10 minutes at ambient temperature. Centrifuge sample mixture at 20 000 x g for 5 minutes to separate visible clots and fats. Transfer 100 µL of the clear supernatant into the well in the microplate according to table 1 for EPO semi quantification.

Immunopurified EPO samples

EPO and especially EPO isoforms often occur at very low concentration together with numerous other molecules in urine, serum, plasma or blood. Therefore, it is often necessary to purify and concentrate EPO before analysis. EPO Purification Kit from MAIIA Diagnostics could be used for rapid purification and concentration of endogenous (hEPO) or recombinant erythropoietin (rhEPO) from aqueous media and intended as a pre-step for further analysis.

- **EPO purification Kit (1390)** has capacity for 20 mL urine or 1 mL EDTA-Plasma or Serum Samples.
- **EPO Purification Gel Kit for Urine (1410)** has capacity for 10 mL urine.
- **EPO Purification Gel Kit for Blood (1430)** has capacity for 0.5 mL EDTA-Plasma or Serum Samples or 100 µL Dried Blood spots (DBS) sample.

Purify sample according to Directions for use from respectively EPO Purification Kit. Final volume is 30-55 µL of purified and concentrated EPO in buffer. Depending on your requirement and application, transfer 1-5 µL of purified sample and 100 µL sample buffer into a microtube. Vortex gently, or prepare the sample mixture directly in the microplate according to table 1. Mix the sample by pipetting up and down a few times.

PROCEDURE

Testing conditions: Temperature +20 to +25°C. Humidity 10 to 80 RH%.

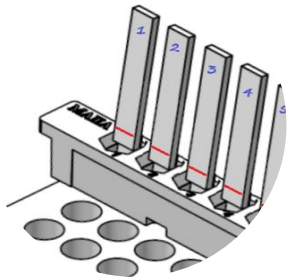
1. Vortex Anti-EPO CBNS before use. Add 1 part of Anti-EPO CBNS to 1 part of CBNS dilution buffer. Vortex this Anti-EPO CBNS working solution before use. This working solution should be used within 3 hours. 25 µL is needed per sample. Use within the same day. Discard after use.

2. Dispense Sample buffer, samples mixture or EPO references, Anti-EPO CBNS working solution and washing buffer into the wells in the microplate. Volume for each buffer is shown in table 1.

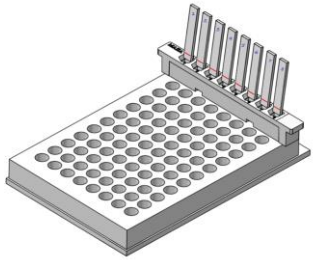
Microplate	Sample volume	Incubation time
Row 1	25 µL Sample buffer	5 min
Row 2	100 µL Sample mixture or EPO reference	20 min
Row 3	25 µL Anti-EPO CBNS working solution	5 min
Row 4	25 µL Washing buffer	5 min

Table 1. Sample volumes and incubation time.

3. Mark each strip with an ID on the absorbent pad according to the sample list and place the strips into the strip holder with the colour code facing front.



4. Immerse the thin end of the strips into the sample buffer and let incubate. Move on to the sample mixture, the Anti-EPO CBNS working solution and finally to the washing buffer. Incubation time for each step is shown in table 1.



5. Remove the protective slip on the Scanning template and mount the strips with the colour code facing up. Remove the absorbent pads and adhesive tape from the strips using forceps. Let the strips dry at room temperature in a draft-free environment for 15 min before comparing the signals against the references by eye.

APPENDIX

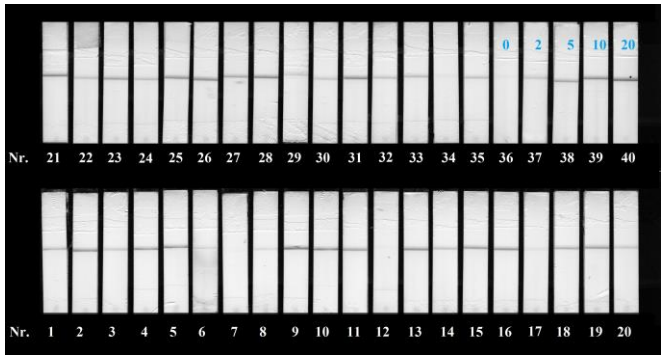


Figure 1. 35 urine samples (10 mL) were purified by using EPO Purification Gel kit for Urine (1410). Strip nr 1-35: 5 µL out of 50 µL purified sample was used for EPO semi quantification. Strip nr 36-40: EPO references with 0, 2, 5, 10 and 20 pg Neorecomon.

WARRANTY

Information presented here is accurate to the best of our knowledge. It is the responsibility of the user to verify the suitability of the supplied materials and procedures for a particular purpose. In this respect, further processing made by the user may affect the results, in which event MAIIA AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. MAIIA AB and its authorised distributors, in such event, shall not be liable for damages indirect or consequential.

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