EPO Purification Gel Kit – For Blood

Directions for Use, 101400/ 06

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INTENDED USE

EPO Purification Gel Kit is used for rapid purification and concentration of endogenous (hEPO) or recombinant erythropoietin (rhEPO) from serum / plasma or dried blood spot (DBS) sample and is intended as a pre-step for further analysis. The kit is designed for single use and to be used in laboratory only.

SUMMARY AND EXPLANATION

In blood, serum and plasma, erythropoietin (EPO) and especially EPO isoforms often occur at a very low concentration together with numerous other molecules. Therefore, it is often necessary to purify and concentrate EPO before analysis with techniques such as SARCOSYL polyacrylamide gel electrophoresis (SAR-PAGE).

PRINCIPLE OF THE PROCEDURE

Buffer is added to serum or plasma samples and after filtration the sample mixture is transferred to the disposable Anti-EPO gel column containing resin with immobilized anti-EPO antibodies for end over end incubation. DBS samples on filter paper or other blood absorbing filters are treated in the same way. EPO is extracted and purified by end over end rotation. The anti-EPO antibody captures both hEPO and rhEPO such as Epoetins, NESP, CERA and EPO-Fc. After purification, aqueous media is removed by pressure format. The affinity resin is washed and the bound EPO is released by Elution buffer. EPO is then highly purified and concentrated in 0.5 % SARCOSYL, 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 0.02 % NaN3, 0.1 % TWEEN 20 with or without 0.01% casein as a protection agent. The purified sample should be stored at -20˚C until analysis.

REAGENTS

Art No	Name and Contents		
1430	EPO Purification Gel Kit - For Blood		
	Contains reagents for 25 tests.		
	Contents:		
	1x Anti EPO gel column, 25 pcs	Ready for use	101410
	1x Sample buffer, 125 mL [a]	Ready for use	101480
	1x Washing buffer, 175 mL[a]	Ready for use	101370
	1x Elution buffer, 5 mL[a]	Ready for use	101471
	1x Elution buffer C (incl. casein), 5 mL[a]	Ready for use	101560

[a] Contains < 0.1 % sodium azide

Storage and Shelf Life

Store all components at +4-8˚C. Do not freeze 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 bodily fluid must be handled and treated as a potentially infectious agent.
- 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.

MATERIALS

Materials required and available from MAIIA Diagnostics:

- Funnel Pack F20, Art No 1420

Equipment and materials required but not provided by MAIIA Diagnostics:

- Vacuum manifold with standardized Luer female taper connection, vacuum source and a regulator to provide a steady vacuum or similar.
- 0.45 µm HPF Millex HV filter (Cat no SLHVM25NS, Millipore) and 20 mL syringe with Luer-Lok.
- 15 mL and 50 mL conical centrifuge tube. Microcentrifuge, tube rotator.

PURIFICATION PROCEDURE

1. Assemble the Anti EPO gel column containing affinity resin with the Funnel F20 and put it into a 50 mL conical centrifuge tube.

2a. Serum / plasma sample preparation.
Add 5 mL Sample buffer and 0.2-0.5 mL sample into a 15 mL conical centrifuge tube and mix gently. Filter the sample mixture through the recommended 0.45 µm HPF Millex HV syringe filter to remove precipitates or cellular debris and add it into the column as illustrated in Fig.1. Prefiltering prevents clogging in the column and reduces background in EPO analysis.

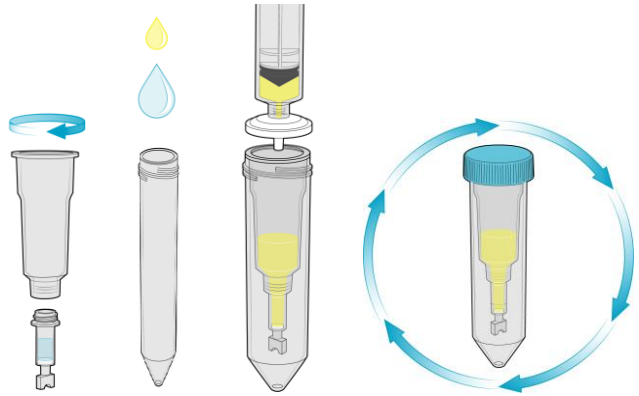

Figure 1. Serum / plasma sample preparation and purification by end over end rotation.

2b. DBS sample preparation.
Add 5 mL Sample buffer and 1-3 DBS filter papers or other similar blood absorbing filters into the column as illustrated in Fig.2.

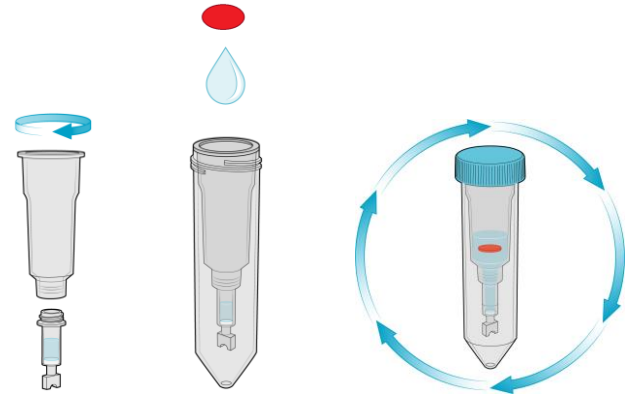

Figure 2. DBS sample extraction and purification by end over end rotation.

3. Seal the funnel/column assembly and incubate the sample mixture with anti-EPO antibodies by continuously rotating end over end using a tube rotator or similar for 90-120 minutes. Choose a proper rotation speed (15-20 rpm) where no sample remains in the column when in the upside-down position.

4. Remove the funnel/column assembly from the tube. Twist off the bottom plug and place the funnel/column assembly on a vacuum manifold as illustrated in Fig.3. Allow the sample to completely pass through the affinity resin by pressure format, approximately at -300 mBar.

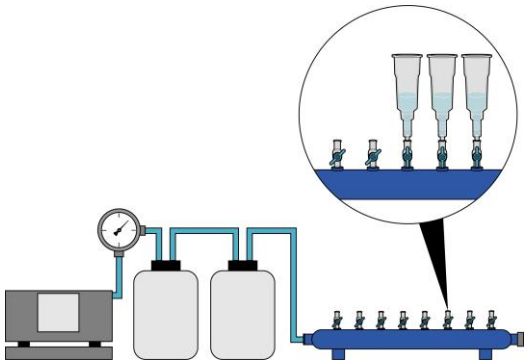

Figure 3. Schematic picture of a vacuum equipment set up. From the left: vacuum source with regulator, tanks to collect waste and vacuum manifold with standardized Luer female taper connection.

5. Close the vacuum valve as soon as the sample has passed through the column and add 5 mL washing buffer. Once you have added washing buffer to all samples, open the valves and let the washing buffer completely pass through the columns.

6. Unscrew the column from the funnel. Make sure there is no DBS filters / blood absorbing filters in the column. Remove it if necessary. Place the column in a microcentrifuge tube and centrifuge for 1 minute at 300 x g to remove any remaining liquid. Discard the tube with the flow through.

7. Place the column in a new microcentrifuge collection tube. Add 35-50 µL Elution buffer or Elution buffer C into the affinity resin and elute the purified EPO by gravity-flow for 5 minutes. Then centrifuge the column with the collection tube for 1 minute at 300 x g to collect the remaining eluate.

Proceed with analysis or store purified samples at -20˚C until analysis. EPO might be degraded in other conditions. Discard the used Anti-EPO column.

Note! Buffer composition in Elution buffer is 0.5 % Sarcosyl, 0.1 M Bis-tris, pH 7.0, 0.1 M NaCl, 0.02 % NaN3 and 0.1 % TWEEN 20. Buffer composition in Elution buffer C is the same but including 0.01 % casein as a protection agent and is recommended for long term storage. If a higher recovery and / or more concentrated purified sample is required, increase the Elution buffer or Elution buffer C volume to 150 µL and elute the purified EPO in the same way. Concentrate the eluate down to 15-20 µL by using a 10-30 kDa centrifugal cut off filter, typically spin for 45 minutes at 14 000 x g.

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.

MANUFACTURER

MAIIA AB, Virdings Allé 22, SE-75450 Uppsala, Sweden.
Web: www.maiiadiagnostics.com
Email: info@maiiadiagnostics.com
Mail: MAIIA Diagnostics, PO Box 6529, SE-75138 Uppsala, Sweden