EPO Purification Gel Kit – For Blood

Directions for Use, 101400/ 05
Issued: Nov 2018, Revised: Oct 2019

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 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

Serum, plasma or DBS samples is added to buffer and after filtration, the sample mixture is added to the disposable Anti-EPO gel column containing resin with immobilized anti-EPO antibodies. The anti-EPO antibody captures both hEPO and rhEPO such as Epoetins, NESP, CERA and EPO-Fc. After washing, the bound EPO is then released by Elution buffer. EPO is then highly purified and concentrated in 35-50 µL 0.5 % SARCOSYL, 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 0.02 % NaN3, 0.1 % TWEEN 20. 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, 1.5 mL[(a)]	Ready for use	101470

[(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 body 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
- Dried Blood Spot Collection Kit, Art No 1440

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. Bring serum or plasma samples and Sample buffer to room temperature.

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

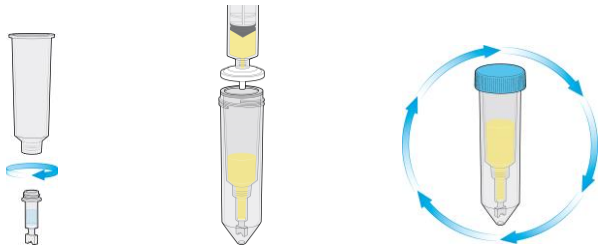

Figure 1. Assembling of Anti-EPO gel columns and funnel.

3. Sample preparation.

- Serum / plasma sample:
  Add 0.2-0.5 mL serum or plasma sample and 5 mL Sample buffer into a 15 mL conical centrifuge tube and vortex gently. Filter the sample mixture through a 0.45 µm HPF filter and add it to the column. Seal the tube and incubate the sample mixture by rotating end over end for approximately 60 minutes at ambient temperature using a tube rotator or similar.

- DBS sample:
  Add 1-2 DBS discs and 5 mL Sample buffer into the column. Seal the tube. Then extract the EPO and purify by rotating the sample mixture end over end for approximately 90 minutes at ambient temperature using a tube rotator or similar.

Note! Choose a proper rotation speed where no sample remains in the column when in the upside-down position.

4. Remove the funnel/column assembly from the tube. Grip it with your non-dominant hand and twist off the bottom plug clockwise with your dominant hand. Place the funnel/column assembly on a vacuum manifold with standardized Luer female taper connection or similar. For DBS sample, remove the DBS disk with a tweezer. Allow the sample completely pass through the affinity resin by pressure format. At -300 mBar all samples should have passed within 1 minute. If not, increase the vacuum level.

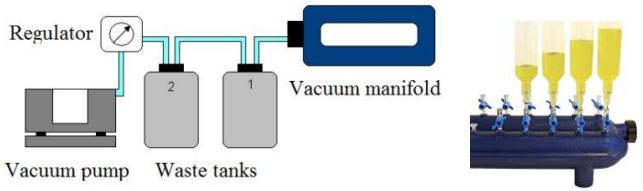

Figure 2. Schematic picture of a vacuum equipment set up.

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. Or, for more effective washing, plug the column bottom with parafilm and rinse the affinity resin with 5 mL Washing buffer by rotating end over end in the same set up as for sample incubation, for approximately 5 minutes. Let the washing buffer completely pass through the columns.

6. Disconnect the column from the funnel and place the column in a microcentrifuge tube and centrifuge for 1 minute at 500 x g to remove remaining liquid. Discard the tube and the flow through.

7. Place the column in a new microcentrifuge tube. Add 35-50 µL Elution buffer into the affinity resin. Let stand for 5 minutes and then centrifuge the column for 1 minute at 500 x g to elute the purified EPO.

8. Collect the microcentrifuge tubes with eluate containing EPO and seal the tube with the push cap. Proceed with analysis or store purified samples at -20˚C until analysis. EPO might be degraded in other conditions. Discard the used and disposable Anti-EPO column.

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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