EPO Purification Gel Kit

Directions for Use, 101350/ 03
Issued: Aug 2018, Revised: April 2019

INTENDED USE

EPO Purification Gel Kit is used for rapid purification and concentration of endogenous (hEPO) or recombinant erythropoietin (rhEPO) from urine sample and intended as a pre-step for further analysis. Designed for single use and to be used in laboratory only.

SUMMARY AND EXPLANATION

Erythropoietin (EPO) and especially EPO isoforms often occur at very low concentration together with numerous other molecules in urine. 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

Precipitates are frequently found in urine samples, especially in acidic samples or after thawing frozen samples. These urine precipitates may contain EPO, therefore a maintained proportion of solid/liquid matters for preparation is crucial when transferring from the original stock sample. Buffer is added to the sample to dissolve most of the precipitates and after filtration the sample mixture is added to the disposable Anti-EPO gel column containing affinity 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 50 µL 0.5 % SARCOSYL, 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 0.02 % NaN3, 0.1 % TWEEN 20, 0.01 % BSA. The purified sample should be stored at -20˚C until analysis.

REAGENTS

Art No	Name and Contents		
1410	EPO Purification Gel Kit – For urine sample		
	Contains reagents for 25 tests.		
	Contents:		
	1x Anti EPO gel column, 25 pcs	Ready for use	101390
	1x Sample Buffer, 30 mL [(a)]	Ready for use	101360
	1x Washing buffer, 175 mL[(a)]	Ready for use	101370
	1x Elution buffer, 2 mL [(a)]	Ready for use	101380

[(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 1320

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. Thaw urine samples if necessary, e.g. using a luke warm water bath.

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.

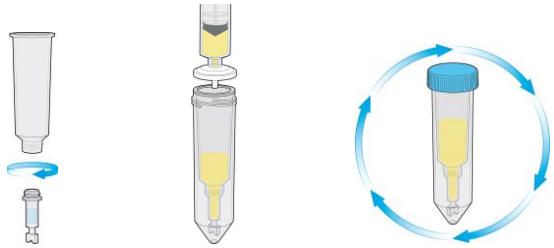

Figure 1. Assembling of Anti-EPO gel columns and funnel.

3. Add 10 mL urine sample and 1 mL Sample buffer into a 15 mL conical centrifuge tube and vortex gently. Let the precipitates settle down for approximately 10 minutes at ambient temperature.

4. If the sample mixture is clear, then transfer the supernatant to the column with a pipette. Otherwise, filter the supernatant 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 90 minutes at ambient temperature using a tube rotator or similar.

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

4. Remove the funnel with column from the tube. Then remove the twist-off bottom of the column and place the column with funnel on a vacuum manifold with standardized Luer female taper connection or similar. 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.

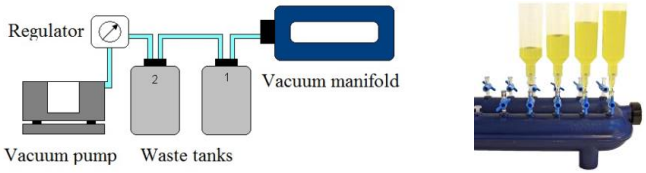

Figure 2. Schematic picture of a vacuum equipment set up.

5. Close the vacuum valve as soon as the sample has passed through their columns and add 5 mL Washing buffer. Once all samples have passed, open the valves and 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 waste.

7. Place the column in a new microcentrifuge tube. Add 50 µL Elution buffer directly into the affinity resin and let incubate in ambient temperature for 10 minutes. Then centrifuge the column for 1 minute at 500 x g to release bound 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. 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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