EPO Purification Gel Kit – For Urine

Directions for Use, 101350/11

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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 urine samples 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 a urine sample, erythropoietin (EPO) and especially EPO isoforms often occur at very low concentrations together with numerous other molecules. Therefore, it is often necessary to purify and concentrate EPO before analysis with techniques such as SARCOSYL (SAR) or sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (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 the proportion of solid to liquid matter should be maintained during preparation when transferring from the original stock sample. Buffers are 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 resin with immobilized anti-EPO antibodies. The anti-EPO antibody captures both hEPO and rhEPO such as Epoetins, NESP, CERA and EPO-Fc. After incubation and washing, 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 0.01% bovine serum albumin (BSA) or 0.01% casein as a protection agent. The purified sample should be stored at -20˚C until analysis.

REAGENTS

Art No	Name and Contents		
1410	EPO Purification Gel Kit – For Urine		
	Contains reagents for 25 tests.		
	Contents:		
	1x Anti EPO gel column, 25 pcs	Ready for use	101390
	1x Buffer for urine, 20 mL[(a)]	Stock solution	101301
	1x Exposure aid, 20 mL [(a)]	Stock solution	101241
	1x Washing buffer, 175 mL[(a)]	Ready for use	101370
	1x Elution buffer B (incl. BSA), 5 mL[(a)]	Ready for use	101381
	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 to +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

Equipment and materials required but not provided by MAIIA Diagnostics:

- Vacuum manifold with standardized Luer female taper connection and vacuum source as illustrated in Fig.3.
- 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.
- Tube rotator and microcentrifuge.

PURIFICATION PROCEDURE

1. Thaw urine samples, e.g. using a luke warm water bath, and bring it to room temperature if necessary.

2. Add 10 mL urine sample, 0.5 mL Buffer for Urine and 0.5 mL Exposure aid into a 15 mL conical centrifuge tube and mix gently.

3. 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.

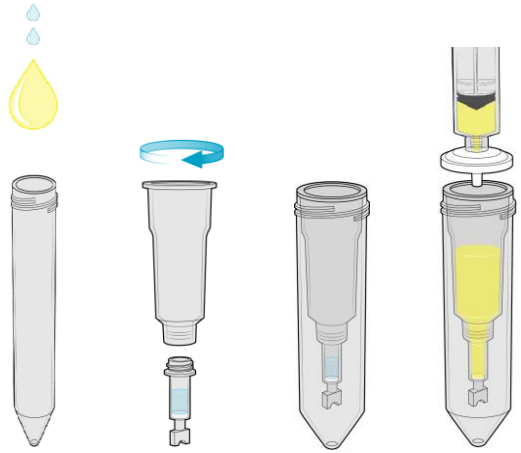

Figure 1. Sample preparation.

4. 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. Prefiltering prevents clogging in the column and reduces background in EPO analysis.

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

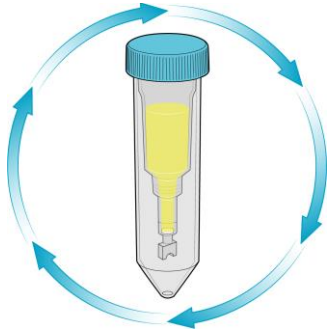

Figure 2. Sample incubation by end over end rotation.

6. 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, typical at -300 mBar.

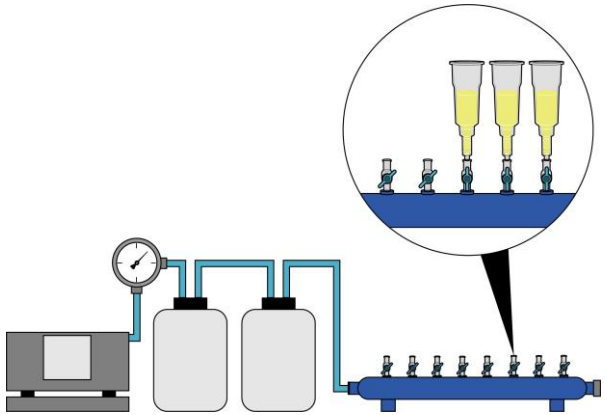

Figure 3. Schematic picture of a vacuum equipment set up. From the left: vacuum source with regulator, tanks to collect waste and vacuum manifold with standardized Luer female taper connection.

7. 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.

8. Disconnect the column from the funnel and place the column in a microcentrifuge tube and centrifuge for 1 minute at 300 x g to remove remaining liquid. Discard the tube with the flow through.

9. Place the column in a new microcentrifuge collection tube. Add 50 µL Elution buffer B or 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 B is 0.5 % Sarcosyl, 0.1 M Bis-tris, pH 7.0, 0.1 M NaCl, 0.02 % NaN3, 0.1 % TWEEN 20 including 0.01 % BSA as a protection agent. In Elution buffer C, BSA is replaced with 0.01% casein and is recommend for EPO analysis with SAR PAGE.

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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