EPO Purification Kit

Directions for Use, 101260/ 07
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INTENDED USE

EPO Purification Kit is used for rapid purification and concentration of endogenous (hEPO) or recombinant erythropoietin (rhEPO) from aqueous media such as urine, serum or EDTA-plasma 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

Erythropoietin (EPO) and especially EPO isoforms often occur at very low concentration together with numerous other molecules in urine, serum and plasma. Therefore, it is often necessary to purify and concentrate EPO before analysis with techniques such as isoelectric focusing (IEF), SARCOSYL (SAR) or sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) and Membrane Assisted Isoform ImmunoAssay (MAIIA).

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 for urine and MilliQ water are added to the urine sample to dissolve the precipitates. Exposure aid is added to enhance the interaction between pegylated EPO e.g CERA and the antibodies on the Anti-EPO column but also preventing unspecific binding to filter and surfaces. Tamm-Horsfall glycoprotein (THP) is a protein commonly found in urine which easily aggregates to macromolecules. If use of urine sample volume exceeds 10 mL, then the samples need to be heated in a boiling water bath to change the THP macromolecule structure thus prevent clogging, and thereby facilitating the flow through the narrow pores in the Anti-EPO columns. Some EPO variants or parts of the modified EPO structure e.g. EPO- Fc might be sensitive to heat. Heat treatment is optional if urine sample volume is less than 10 mL.

Fibrinogen and high protein content in the serum/plasma samples may occasionally obstruct the pores of the Anti-EPO column and affect the flow rate through the column. This issue is not frequently found if the applied sample amount does not exceed 1 mL. Buffer for plasma or serum, Exposure aid and MilliQ water are added to the sample for the same reasons as urine sample.

The disposable Anti-EPO column with immobilized monoclonal anti-EPO antibody captures very specifically both hEPO and rhEPO from urine, serum or plasma. The bound EPO is then released by either the use of 0.5% SARCOSYL in pH neutral buffer or by an acidic buffer which pH adjust to neutral later. EPO is then highly purified and concentrated with preserved isoform distribution in 35-50 µL eluate. The eluted sample should be stored at -20˚C until analysis.

REAGENTS

Art No	Name and Contents		
1390	EPO Purification Kit		
	Contains reagents for 25 tests.		
	Contents:		
	1x Anti-EPO column, 25 pcs	Ready for use	101220
	1x Buffer for urine, 30 mL [a]	Stock solution	101300
	1x Buffer for plasma or serum, 30 mL [a]	Stock solution	101250
	1x Exposure aid, 30 mL [a]	Stock solution	101240
	1x Washing buffer, 30 mL[a]	Ready for use	101280
	1x Elution buffer A (acidic), 5 mL [a], [b]	Ready for use	101570
	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
	1x Adjustment buffer A, 0.5 mL[a]	Ready for use	100604
	1x Adjustment buffer B, 0.5mL[a]	Ready for use	100951

[a] Contains < 0.1 % sodium azide
[b] Contains < 0.2 % hydrochloric acid

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 F40, Art No 1340

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), 50 mL syringe with Luer-Lok, 50 mL conical polypropylene centrifuge tube, 1.5 mL microcentrifuge tube and microcentrifuge.

PREPARATION OF SAMPLES

EDTA-plasma or Serum Samples

1. Bring EDTA-plasma or serum samples to room temperature.

2. Transfer 0.5-1.0 mL sample into a 50 mL conical tube. Add 20 mL MilliQ water, 1 mL Buffer for plasma or serum and 1 mL Exposure aid to the sample. Mix gently and let incubate in ambient temperature for approximately 10 minutes. Proceed with purification.

Urine Samples

1. Bring urine samples to room temperature, e.g., using a lukewarm water bath.

2. Prepare a boiling (100 ˚C) water bath. The water level must reach above the sample level.

3. Transfer 15-20 mL of representable urine sample from original stock into a 50 mL polypropylene conical tube. Seal the cap lightly to preventing pressure build up and boil it for 5 minutes. Thereafter, immediately cool the sample in a chilled water bath for approximately 10 minutes.

4. Add MilliQ water to the sample and fill to 40 mL. Then add 1 mL Buffer for urine and 1 mL Exposure aid to the sample. Mix gently and let incubate for approximately 10 minutes. Proceed with purification.

Note!

Urine sample volumes that exceed 10 mL need to be heated in a boiling water bath to prevent clogging in the Anti-EPO column. Some EPO variants or parts of the modified EPO structure e.g., EPO-Fc might be sensitive to heat and thus not recognized by the antibody in the Anti-EPO column. However, heat treatment is optional for urine sample volumes less than 10 mL and clogging is only affected by for a few urine samples, if not heated. Reduce buffers and MilliQ water by half if the sample volume is less than 10 mL.

PURIFICATION PROCEDURE

1. Connect the vacuum equipment according to the manufacture handbook. Carefully check that no connections or parts are broken. If necessary, replace broken parts before proceeding.

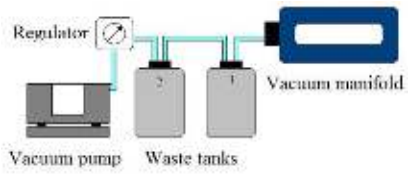

Figure 1. Schematic picture of a vacuum equipment set up.

2. Place the Anti-EPO column/funnel assembly on a vacuum manifold with standardized Luer female taper connection as illustrated in Fig.2. Close all unused slots.

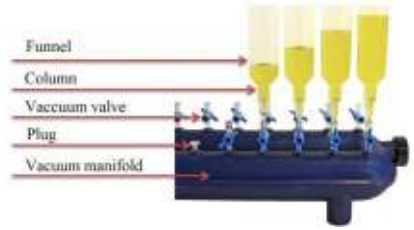

Figure 2. Assembling of Anti-EPO columns and funnels on vacuum manifold.

3. Start with a low vacuum level suitable for your equipment, for example 50-100 mbar (~5 kPa) below normal pressure.

4. Add 1 mL Washing buffer to one of the Anti-EPO columns. Open the valves and make sure that the flow rate is approx. 1.0 mL/min. If necessary, repeat step 4 and adjust the vacuum level.

5. Filter the sample mixture through the recommended 0.45 µm HPF HPF Millex HV syringe filter and add it into the anti-EPO column. Let the sample completely pass through the Anti-EPO column.

Note!
Check the flow rate at every 10 minutes. If any sample shows a considerably lower flow rate, increase the vacuum level. If this still does not work even at maximum vacuum levels, then filter the remaining sample mixture through a 0.45 µm HPF filter and run the filtrate on a new Anti-EPO column.

6. Close the vacuum valve as soon as the sample has passed through their columns and add 1 mL Washing buffer. Once all samples have passed, open the valves and let the washing buffer completely pass through the columns.

7. Remove the Anti-EPO column from the vacuum manifold. Place it in a new micro tube and centrifuge for 1 minute at 2000 x g to remove remaining liquid. Discard the tube and the waste.

8a. Recommendation for IEF and MAIIA analysis:
Add 5 µL Adjustment buffer A or B into a new microcentrifuge collection tube and place the Anti-EPO column in the tube. Add 50 µL of Elution buffer A (acidic) directly into the Anti-EPO column and centrifuge the column for 1 minute at 2000 x g to release bound EPO. Collect the collection tubes with eluate containing EPO and mix gently to bring back the pH to neutral immediately. Proceed with analysis or store at -20˚C until analysis. Discard the used Anti-EPO column.

Note!
Use Adjustment buffer A if analysis require absent of detergent and protection protein. The final buffer composition will be: 55 µL 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 10 mM Glycine, 0.02 % NaN3. If using Adjustment buffer B, the final buffer composition will also include: 0.02 % TWEEN 20, 0.05 % BSA and is more suitable for long term storage. A mix of Adjustment buffer A and B can also be used to adjust the TWEEN and BSA concentration. At least 0.01% BSA is recommended as protection agent and carrier protein for detection of CERA in IEF gel.

8b. Recommendation for SAR-PAGE analysis:
Place the anti-EPO column in a new microcentrifuge collection tube. Add 35 µL Elution buffer B or C into the Anti-EPO column and let incubate in ambient temperature for approximately 5 minutes. Then centrifuge the column for 1 minute at 2000 x g to release bound EPO. Collect the collection tubes with eluate containing EPO. Proceed with analysis or store purified samples at -20˚C until analysis. 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. Buffer composition in Elution buffer C is the same with the following changes: BSA is replaced with 0.01% casein. A mixture of Elution buffer B and C can also be used to adjust the BSA/casein concentration. Adjustment buffer is not needed.

APPENDIX

9 serum samples (0.5 mL) and 9 urine samples (10 mL, not heated) were spiked with 40 pg BRP, 13 pg NESP, 125 pg EPO-Fc and 200 pg CERA each. Samples were purified and eluted with 35µL Elution buffer B. All purified samples were pooled and then re-aliquoted. 1 replicate was placed in the freezer and another replicate was placed in the fridge as a constant. 5 replicates went through 1-5 freeze/thaw cycles which involved being left in room temperature for 1 h, then placed back into the freezer for 45 minutes to re-freeze. 1 blank urine and 1 serum sample was purified and placed in the freezer. Analysis was made by SAR-PAGE; single blotting, 8µL purified sample + 2µL loading buffer.

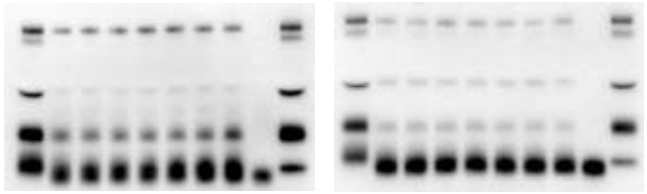

Figure 3. Unmodified pictures of the gels. Serum samples (left picture) and urine samples (right picture). Positions from left; ESA standard (BRP, NESP, EPO-Fc, CERA), 1 to 5 freeze/thaw cycles, -20 ˚C, + 4 ˚C, blank sample and ESA standard.

WARRANTY

TRADEMARKS

MANUFACTURER

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