EPO Purification Kit

Directions for Use, 101260/ 04

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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 intended as a pre-step for further analysis. To be used in laboratory only.

SUMMARY AND EXPLANATION

Erythropoietin (EPO) often occurs 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 immunochemical techniques or LC/MS. This is particularly true when determining the EPO isoforms, which occur at even lower concentrations with methods such as IEF, PAGE and MAIIA.

PRINCIPLE OF THE PROCEDURE

Urine precipitates frequently found in the samples may contain EPO. A maintained proportion of solid/liquid matters for preparation is crucial when transferring from original stock sample. Buffers are added to urine samples as well as to serum and plasma samples to enhance the interaction between EPO and antibody on the Anti-EPO column. Tamm-Horsfall glycoprotein (THP) is a protein commonly found in urine which easily aggregates to macromolecules. Heating the samples in a hot water bath will change the THP macromolecule structure thus preventing clogging and facilitate the flow through the narrow pores in the Anti-EPO columns.

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 the use of an acidic buffer and thereafter neutralized with an adjustment buffer to a final volume of 55 µL eluted sample. EPO is now highly purified and concentrated with preserved isoform distribution in 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 10 mM Glycine, 0.02 % NaN3 with or without 0.1 % TWEEN 20 and 0.05 % BSA. The buffer composition containing TWEEN 20 and BSA is recommended for isoform analysis using MAIIA or long term storage. 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 Dummy column (red), 1 pce	Ready for use	100542
	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 Desorption buffer, 5 mL [a], [b]	Ready for use	100270
	1x Adjustment buffer A, 0.5 mL[a]	Ready for use	100604
	1x Adjustment buffer B (TWEEN 20, BSA), 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) and 50 mL syringe with Luer-Lok
- Standard laboratory materials/equipment, e.g. 50 mL polypropylene conical tubes, 1.5 or 2 mL Eppendorf micro tube, vortex, MilliQ water, microcentrifuge.

PREPARATION OF SAMPLES

Serum or EDTA-plasma: 1- 2 mL
Urine: 20 mL

EDTA-plasma or Serum Samples

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

2. Transfer 1-2 mL EDTA-plasma or serum sample into a 50 mL conical tube. Add MilliQ water in the sample and fill to 20 mL. Then add 1 mL Buffer for plasma or serum (Art No 101250) and 1 mL Exposure aid (Art No 101240) to the sample. Mix gently and let incubate in ambient temperature for approximately 10 minutes.

3. Filter the sample mixture through a 0.45 µm HPF filter to a new conical tube. If the same conical tube is being reused, make sure that the tube is rinsed with water before filling with filtered sample. Proceed with purification steps.

Urine Samples

1. Bring urine samples to room temperature.

2. Prepare a hot (95-100 ˚C) water bath. The water level must reach above the sample level.

3. Transfer 10-20 mL urine sample with a preserved proportion of solid/liquid matters as in the original stock sample into a 50 mL polypropylene conical tube. Close the tube with the cap.

4. Place the tube with the sample in the hot water bath. Heat for **5 minutes** and keep the temperature constant during the heating procedure. Thereafter, **immediately** cool the sample in a cold (15-25 ˚C) water bath for approximately 10 minutes.

Warning! The pressure in the tubes may increase as a result of the heating. Do not shake and do not open the tube directly after heating!

5. After cooling, add MilliQ water in the sample and fill to 40 mL. Then add 1 mL Buffer for urine (Art No 101300) and 1 mL Exposure aid (Art No 101240) to the sample. Mix gently and let incubate in ambient temperature for approximately 10 min.

6. Filter the sample mixture through a 0.45 µm HPF filter to a new conical tube. If the same conical tube is being reused, make sure that the tube is rinsed with water before filling with filtered sample. Proceed with purification steps.

PURIFICATION PROCEDURE

Assembling of the Vacuum Equipment

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.

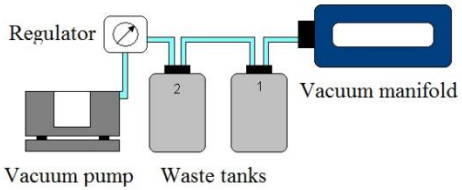

Figure 1. Schematic picture of a vacuum equipment set up.

2. Place the vacuum valve, the Dummy column (Art No 100542) and the Funnel (Art No 1340) on the vacuum manifold. Close all unused slots. The Dummy column is used for control of vacuum equipment and flow rate adjustment only. It is not intended for capturing of any specific molecules.

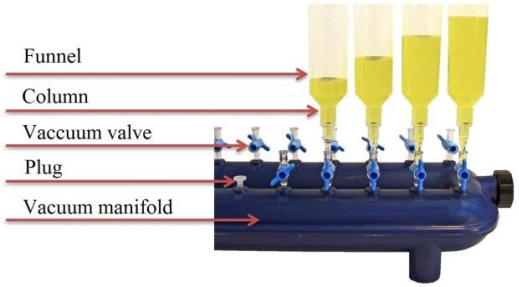

Figure 2. Assembling of Anti-EPO columns and funnels on vacuum manifold.

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

4. Add 20 mL of water to the graduated funnel, which is marked at every 10 mL level. Open the valves, measure the time for 10 mL of water to pass through the column and thereafter calculate the flow rate. Make sure that the flow rate is approx. 1.0 mL/min. If necessary, repeat step 4 and adjust the vacuum level.

Purification of EPO from Pre-treated Plasma, Serum or Urine Samples

1. Mark the Anti-EPO column (Art No 101220) and the collecting 1.5 mL micro tube with the sample ID. Place the vacuum valve, the marked Anti-EPO column, and the funnel on the vacuum manifold. Close all unused slots.

2. Add the pre-treated sample to the funnel. Start the vacuum source and let the sample completely pass through the Anti-EPO column. The flow rate should be approx.1.0 mL/min. Close the vacuum valve when the sample has passed through their columns and add 1 mL Washing buffer (Art No 101280).

If any sample shows a considerably lower flow rate, increase the vacuum level after the other samples have passed. Or add an additional 1 mL Buffer for urine (Art No 101300) to the remaining urine sample mixture. Filter it through a 0.45 µm HPF filter and run the filtrate on a new Anti-EPO column (Art No 101220). For plasma or serum samples, just filter the remaining sample mixture through a 0.45 µm HPF filter and run the filtrate on a new Anti-EPO column (Art No 101220).

3. Once all samples have passed, open the valves and let the washing buffer completely pass through the columns.

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

5. Add 5 µL Adjustment buffer to the ID marked collecting micro tube and place the Anti-EPO column with the same ID in the tube.

- Use Adjustment buffer A (Art No 100604) if TWEEN 20 and BSA are **not** desired in the purified EPO elutent.
- Use Adjustment buffer B (Art No 100951) if TWEEN 20 and BSA are desired in the purified EPO elutent.

6. Add 50 µL of Desorption buffer (Art No 100270) directly into the Anti-EPO column. **Immediately**, centrifuge the column for 1 min at 2000 x g to release bound EPO.

7. Collect the collecting micro tubes with eluate containing EPO and vortex gently. Proceed with analysis or store at -20˚C until analysis.

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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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