EPO Purification Gel

Directions for Use, 100860/05 (EN)
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INTENDED USE

EPO Purification Gel is used for rapid purification and concentration of endogenous (hEPO) or recombinant erythropoietin (rhEPO) from aqueous media such as urine, serum, EDTA-plasma or cellculture medium and is intended as a pre-step for further analysis. To be used in laboratories only.

SUMMARY AND EXPLANATION

Erythropoietin (EPO) often occurs at very low concentrations 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.

Anti-EPO gel is specially designed for EPO purification when a large sample volume with high EPO concentration is used. The Anti-EPO gel is regenerable and characterized by high EPO recovery and retained isoform distribution.

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.

The Anti-EPO gel immobilized with monoclonal anti-EPO antibody captures very specifically both hEPO and rhEPO from urine, serum, plasma or cellculture medium. The bound EPO is then released by the use of an acidic buffer and thereafter neutralized with an adjustment buffer. The final volume of the eluted sample is 2-3 times that of the gel volume. 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 % NaN_3 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 time storage. The eluted sample should be stored at -20˚C until analysis.

REAGENTS/CONTENTS

Art. No.	Name and Contents		
1271	EPO Purification Gel		
	Contents:		
	1x Anti-EPO gel column, 2 mL [a], [c]	Stock solution	100631
	1x Tubing and adapter		990098
	2x Buffer for urine, 30 mL [a]	Stock solution	101300
	2x Exposure aid, 30 mL [a]	Stock solution	101240
	2x Buffer for plasma or serum, 30 mL [a]	Stock solution	101250
	2x Desorption buffer, 30 mL [a], [b]	Ready for use	100273
	2x Adjustment buffer A, 1mL[a]	Ready for use	100603
	2x Adjustment buffer B (TWEEN 20, BSA), 1mL[a]	Ready for use	100950

[a] Contains < 0.4 % sodium azide
[b] Contains < 0.2 % hydrochloric acid
[c] Binding capacity: 150- 400 µg EPO depending on EPO variant.

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

- EPO Purification Gel Running buffers (Art No 1300, MAIIA Diagnostics) for reuse of the Anti-EPO Gel.
- 0.45 µm HPF Millex HV filter (Cat no SLHVM25NS, Millipore) or GMF 150 – 1 µm, multilayer glass microfiber filter (Cat no 1841-047, Whatman) and 0.45 µm HVLP filter (Cat no HVLP04700, Millipore),

PREPARATION OF SAMPLES

1. Bring all reagents and samples to room temperature.

2. Prepare a working sample dilution buffer. Add 10 mL Buffer for plasma or serum (Art No 101250) and 10 mL Exposure aid (Art No 101240) to 200 mL MilliQ water. Mix gently and degas.

3. Prepare samples.

- EDTA-plasma or serum: up to 20 mL
  Transfer the plasma or serum sample into a suitable glass vessel and dilute the sample to 5-10% with working sample dilution buffer. Mix gently.

- Urine: up to 1000 mL
  Transfer urine sample with a preserved proportion of solid/liquid matters as in the original stock sample into a suitable glass vessel. Add 1 part Buffer for urine (Art No 101300) and 1 part Expoure aid (Art No 101240) to 20 parts sample. Mix gently.

- Cellculture medium:
  Pre concentrate the sample with suitable cut-off filter by centrifugation to 1-2 ml, if possible. Dilute the concentrated sample to 5-10% with working sample dilution buffer. Mix gently.

4. Pre-filter the sample mixture. For smaller sample volume use the recommended 0.45 µm HPF filter. For larger sample volume, filter the sample mixture first through a 10-1 µm multilayer glass microfiber filter and then through a 0.45 µm filter. Degas the sample mixture before purification.

PURIFICATION PROCEDURE

Important! Never let the column run dry! Plug the Anti-EPO gel column outlet tip to stop the flow between buffer changes.

1. Equilibrate the column.
Add 10 mL working sample dilution buffer to the column (Art No 100631) and allow the buffer to pass through.

2. Apply the sample.
Add the pre-treated sample mixture to the column and allow the sample mixture to pass through.

- For larger sample volumes, apply the Siphon principle and adjust the flow rate by turning the reel in the tubing clamp.
- For small sample volumes, add sample proportion to one column volume, wait 5-10 seconds, then add more sample, and so on.

Recommended flow rate:

- Urine, serum or EDTA-plasma sample 1-3 mL/min
- Sample with high EPO contain 0.5-1 mL/min or add a small portion of sample at a time

3. Wash the column.
Add 10 mL working sample dilution buffer to the column and allow the buffer to pass through.

4. Eluate the sample.
For desorption of 2 mL gel: Add 0.5 mL of Adjustment buffer to a collecting tube and place it under the column.

- Use Adjustment buffer A (Art No 100603) if TWEEN 20 and BSA are **not** desired in the purified EPO eluated sample.
- Use Adjustment buffer B (Art No 100950) if TWEEN 20 and BSA are desired in the purified EPO eluated sample.

Add 5 mL of the Desorption buffer (Art No 100273) to the column and collect the outlet purified EPO into the collecting tube containing Adjustment buffer. Vortex gently. Proceed with analysis or store at -20˚C until analysis.

5. Clean and Re-equilibrate.
Reuse of the Anti-EPO gel column depends on the nature of the samples and should only be considered when processing identical samples to avoid cross-contamination. Desorption buffer is an acidic buffer and should be remove from the column **immediately.** Thereafter, neutralise with Working sample dilution buffer.

Add 10 mL Desorption buffer and allow the buffer to pass through with fast flow rate. Thereafter, add 10 mL Working sample dilution buffer and allow it to pass through. Plug the outlet tip. Thereafter, add 2 mL Working sample dilution buffer to the column and seal the top of the column. Store it at +4˚C until next purification.

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 an event, shall not be liable for damages indirect or consequential.

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