EPO Purification Gel

Directions for Use, 100860/06 (EN)
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INTENDED USE

EPO Purification Gel is used for rapid purification and concentration of recombinant erythropoietin (rhEPO) from aqueous media such as cell culture medium and is intended as a pre-step for further analysis. To be used in laboratories only.

SUMMARY AND EXPLANATION

Erythropoietin (EPO) and EPO isoforms might occur together with numerous other molecules in cell culture medium. Therefore, it is often necessary to purify and concentrate EPO before analysis with techniques such as isoelectric focusing (IEF), SARCOSYL polyacrylamide gel electrophoresis (SAR-PAGE), Membrane Assisted Isoform ImmunoAssay (MAIIA) or LC/MS. Anti-EPO gel is specially designed for EPO purification when a large sample volume or 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

Buffers are added to the sample and after filtration the sample mixture is added to the Anti-EPO gel column containing affinity resin with immobilized anti-EPO antibodies which captures both hEPO and rhEPO such as Epoetins, NESP, CERA and EPO-Fc. After washing, the bound EPO is then released using an acidic buffer and thereafter neutralized with an adjustment buffer. The final buffer composition is 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 10 mM Glycine, 0.02 % NaN3. For long time storage, add protection agent such as 0.05% BSA or similar is recommended. The purified 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, 2 mL [a], [c]	Stock solution	100631
	1x Tubing and adapter	Ready for use	990098
	2x Sample Buffer, 30 mL [a]	Stock solution	101550
	2x Washing buffer, 175 mL[a]	Ready for use	101370
	2x Desorption buffer, 100 mL [a], [b]	Ready for use	100274
	2x Adjustment buffer A, 5 mL[a]	Ready for use	100605

[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

Materials available from MAIIA Diagnostics:

- EPO Purification Gel Running buffers (Art No 1300, MAIIA Diagnostics) for reuse of the Anti-EPO Gel.

COLUMN PACKING PROCEDURE

1. Clamp the empty column with bottom frit vertically.

2. Resuspended the Anti-EPO gel stock solution by turning end over end carefully and pour it down along the inside wall of the empty column. Add 5 mL Washing buffer to the stock solution to rinse the remainder gel and pour it to the column. Fill up the column with Washing buffer and allow the slurry to settle for a few minutes and forming a gel bed.

3. Place one frit in Washing buffer and sonicate for 5 minutes. Place the wet frit into the column. Make sure there is no air beneath and gently press the filter down to the gel bed. Cut the tip of the column and drain the buffer. If the gel bed has been compressed further, press the filter down to the gel bed.

4. Plug the outlet tip and add 5 mL Washing buffer to the column. Seal the top of the column and store it at +4-8˚C.

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. Bring all reagents and samples to room temperature.

2. If sample volume is low, typical 50 mL or less; add 1 part Sample buffer to 20 parts cell culture medium. Mix gently and filter the sample mixture through a 0.45 µm filter. Otherwise, if sample volume is above 50 mL or EPO concentration is low; filter the sample through a 0.45 µm filter and follow by pre-concentration with suitable cut-off filter and concentrate down to 20-50 mL. Add 1 part Sample buffer to 20 parts cell culture medium and mix gently.

3. Drain the buffer in the packed column. Add 10 mL Desorption buffer and allow the buffer to pass through. Attached the tubing with clamp to column outlet tip. Add 10 mL Washing buffer to the column and allow the buffer to pass through at 0.5-1.0 mL/min. Adjust the flow rate by turning the reel in the tubing clamp.

4. Add the sample mixture to the column and allow it to pass through the anti EPO gel with the pre-set flow rate or add a small portion (half of the column volume) of the sample mixture at a time, wait 5-10 seconds, then add another portion of the sample mixture etc.

5. Remove the tubing with a clamp. Add 10 mL Washing buffer to the column and allow the buffer to pass through.

6. Add 0.5 mL of Adjustment buffer A to a collecting tube and place it under the column. Add 5 mL Desorption buffer to the column and collect the eluted purified EPO into the collecting tube containing Adjustment buffer A. Vortex gently to bring the pH back to neutral.

Note: If high concentration of purified sample is desired, concentrate all eluate by using 10 kDa cut off filter or similar. For long time storage, add protection agent such as 0.05% BSA is recommended. The purified sample should be stored at -20˚C until analysis.

7. Desorption buffer is an acidic buffer and should be removed from the column **immediately**. Add 10 mL Washing buffer to the column and allow 8 mL to pass through. Plug the outlet tip and leave 2 mL Washing buffer left in the column. Seal the top of the column and store it at +4-8˚C until next purification. 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.

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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