EPO Purification Gel Kit 3F6
For Cell Culture Medium

Directions for Use, 100860/07 (EN)

Issued: May 2012, Revised: Mar 2026

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), polyacrylamide gel electrophoresis (PAGE) 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.

PRINCIPLE OF THE PROCEDURE

The sample is mixed with buffer. After filtration, the sample mixture is transferred to the Anti-EPO gel column containing resin immobilized with anti-EPO antibodies 3F6. The sample mixture is incubated with the affinity resin by continuously rotating end over end using a tube rotator. After purification, aqueous media is removed. The affinity resin remaining in the column is rinsed with a washing buffer before the bound EPO is 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.

REAGENTS/CONTENTS

Art. No.	Name and Contents		
1470	EPO Purification Gel Kit 3F6 - For Cell Culture Medium		
	Contents:		
	1x Anti-EPO gel column 3F6 [a], [c]	Ready for use	100632
	1x Sample Buffer, 7.5 mL [a]	Stock solution	101551
	1x Washing buffer, 175 mL[a]	Ready for use	101370
	2x Elution buffer A (acidic), 30 mL [a], [b]	Ready for use	101571
	1x Adjustment buffer A, 2 mL[a]	Ready for use	100605

[a] Contains < 0.4 % sodium azide
[b] Contains < 0.2 % hydrochloric acid
[c] Binding capacity: 10 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.
- 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

Equipment and materials required but not provided by MAIIA Diagnostics:

- Tube rotator and microcentrifuge.
- 0.45 µm syringe filter, 20 mL syringe with Luer-Lok.

PURIFICATION PROCEDURE

Important! Never let the column run dry more than a few minutes!

1. Remove the upper cap of the gel column containing affinity resin and assemble the column with the funnel. Place the funnel/column assembly in a 50 mL conical centrifuge tube.

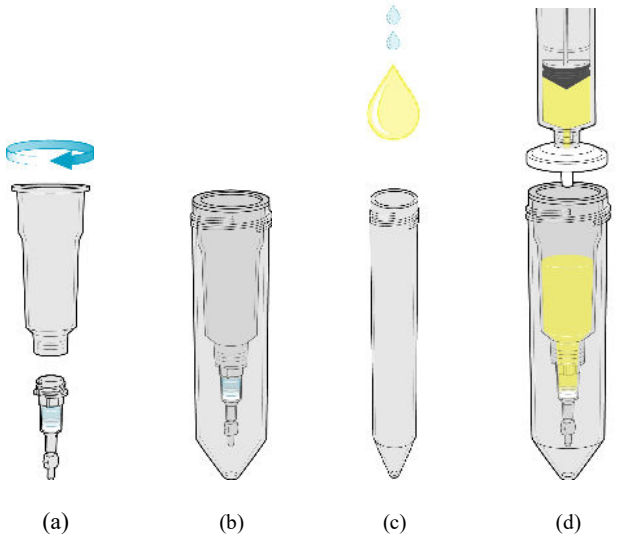

Figure 1. Gel column assembling (a, b). Sample preparation (c, d).

2. Add 0.25 mL of Sample buffer and 0.1 – 0.5 mL of cell culture medium sample into a 15 mL conical centrifuge tube. Fill with milliQ water to 5 mL and mix gently. Filter the sample mixture through a 0.45 µm syringe filter and add the filtrate into the column.

Note: If the starting culture medium sample volume is above 0.5 mL or if the EPO concentration is low, pre-concentration with suitable cut-off filter to 0.1 - 0.5 mL. The total volume in the column must be kept to approximately 5 mL.

3. Secure the top of the 50 mL centrifuge tube and incubate the sample mixture with the affinity resin by continuously rotating end over end using a tube rotator for 2h. Choose a proper rotation speed where no sample remains in the column when in the upside-down position, typically at 15-20 rpm.

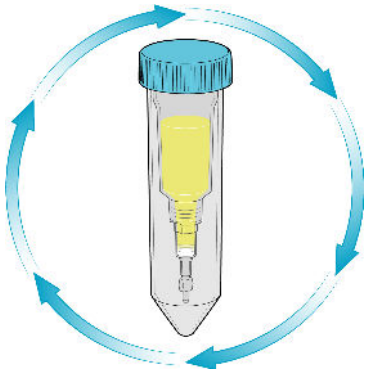

Figure 2. Purification by end over end rotation.

4. Remove the funnel/column assembly with the sample mixture from the 50 mL conical centrifuge tube. Snap off the plug from the bottom of the column and allow the sample mixture to completely pass through the affinity resin. Collect and save the sample flow through.

5. Transfer the funnel/column assembly to a new 50 mL conical centrifuge tube. Add 5 mL of Washing buffer to the column and let the washing buffer completely rinse through the columns. Discard the tube with the flow through.

6. Unscrew the column from the funnel. Place the column in a 1.5 mL tube and centrifuge for 1 minute at 300 x g to remove remaining liquid in the column. Discard the tube with the flow through.

7. Close the outlet of the column with the plug and add 0.3 mL Elution buffer into the column. Close the upper cap and vortex gently for a few seconds. Let incubate for 1 minute.

8. Add 30 µL Adjustment buffer A into a new microcentrifuge collection tube. Remove the upper cap and the plug of the gel column and place the column in the tube. Centrifuge the column for 1 minute at 300 x g to release bound EPO.

9. Collect the collection tubes with eluate containing purified EPO and mix gently to bring back to neutral pH immediately.

10. Repeat step 7, 8 and 9 to collect fraction 2. Regenerate the gel column immediately!

Note: If high concentration of purified sample is desired, concentrate all eluate by using 10 kDa cut off filter. For long term storage, adding a protection agent is recommended.

REGENERATION AND STORAGE

Important! Never let the column run dry for more than a few minutes! The 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.

1. Close the outlet of the column with the plug and add 0.5 mL Elution buffer into the column. Close the upper cap and vortex gently for a few second. Let incubate for 1 minute.

2. Remove the cap and plug. Place the column in a 1.5 mL tube and centrifuge for 1 minute at 300 x g to remove remaining liquid in the column.

3. Repeat step 1 and 2 once with Elution buffer.

4. Repeat step 1 and 2 twice with Washing buffer.

5. Close the outlet of the column with the plug and add 0.5 mL Washing buffer as storage buffer in the column. Seal the top with the cap and store the column at +4 to +8˚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 event, shall not be liable for damages indirect or consequential.

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