# EPO Purification Kit 3F6

**Directions for Use, 101260/ 08**
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## INTENDED USE

EPO Purification Kit 3F6 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

In a urine, serum and plasma sample, erythropoietin (EPO) and especially EPO isoforms often occur at very low concentrations together with numerous other molecules. Therefore, it is often necessary to purify and concentrate EPO before analysis with techniques such as SARCOSYL (SAR) or sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis.

## PRINCIPLE OF THE PROCEDURE

Precipitates are frequently found in acidic urine samples or after thawing frozen urine samples. These urine precipitates may contain EPO, therefore the proportion of solid to liquid matter should be maintained when transferring from the original stock sample. Tamm-Horsfall glycoprotein (THP) is a protein commonly found in urine which easily aggregates to macromolecules. To prevent clogging in the Anti-EPO columns, urine sample could be heated in a boiling water bath. The heating procedure will change the THP macromolecular structure. After cooling, buffers are added to the sample to dissolve most of the precipitates. Sample mixture is filtered and then passed through a disposable Anti-EPO column with an immobilized monoclonal anti-EPO antibody 3F6 by pressure format. The antibody captures hEPO and rhEPO such as Epoetins, NESP, CERA and EPO-Fc. Serum/plasma samples are treated in a similar way, but heat treatment is not needed. Buffers are added to serum or plasma samples. After filtration, the sample mixture is transferred to the disposable Anti-EPO gel column for purification. The column in both cases is rinsed with a washing buffer before the bound EPO is released by either the use of 0.5% SARCOSYL in pH neutral buffer or by an acidic buffer in which the pH is adjusted 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 3F6 | | |
| | Contains reagents for 25 tests. | | |
| | Contents: | | |
| | 1x Anti-EPO column 3F6, 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 to +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 as illustrated in Fig.1.
- 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 pure water (type 2), 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.

3. Filter the sample mixture through the recommended 0.45 µm HPF Millex HV syringe filter to remove precipitates or cellular debris. The filtrate is now ready for purification.

### Urine Samples

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

2. Prepare a boiling (95-100 ˚C) water bath.

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 when incubate the sample in the boiling water bath for 5 minutes. The water level must reach above the sample level.

4. Cool the tube with the sample in a chilled water bath for approximately 10 minutes.

5. Add pure water (type 2) to the sample and fill to 40 mL. Add 1 mL Buffer for urine and 1 mL Exposure aid to the sample. Mix gently and let incubate for approximately 10 minutes.

6. Filter the sample mixture through the recommended 0.45 µm HPF HPF Millex HV syringe filter to remove precipitates or cellular debris. The filtrate is now ready for purification.

**Note!** Urine samples 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, if the sample volume is less than 10 mL, the clogging is only affected for a few urine samples, thus heat treatment is optional. Alternative method: Transfer 10 mL urine sample to a 50 mL polypropylene conical tube. Add 10 mL pure water (type 2), 0.5 mL Buffer for urine and 0.5 mL Exposure aid. Mix gently and let incubate for approximately 10 minutes. Filter the sample mixture through the recommended 0.45 µm HPF HPF Millex HV syringe. The filtrate is now ready for purification.

## PURIFICATION PROCEDURE

1. Connect the vacuum equipment according to the manufacture handbook or as illustrated in Figure 1. Carefully check that no connections, parts and tubings are leaking or broken. If necessary, replace the broken parts before proceeding. Place the Anti-EPO column/funnel assembly on a vacuum manifold. Close all unused slots.

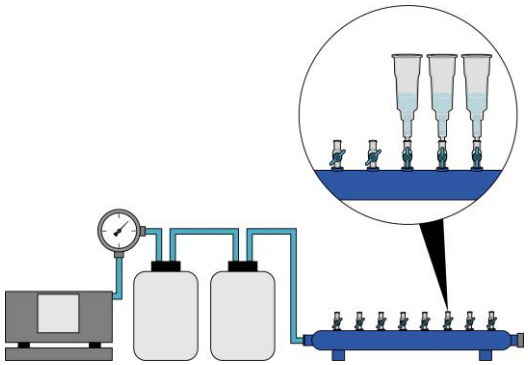

**Figure 1.** Schematic picture of a vacuum equipment set up. Vacuum source with regulator, tanks to collect waste and vacuum manifold with standardized Luer female taper connection. Vacuum valve is placed between funnel/column assembly and vacuum manifold.

2. Start with a low vacuum level suitable for your equipment, typically at -100 mBar. 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, adjust the vacuum level.

3. Add the filtered sample mixture into the anti-EPO column. Let the sample completely pass through the Anti-EPO column.

**Note!** Check the flow rate 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.

4. Close the vacuum valve as soon as the sample has passed through the column. Add 1 mL of Washing buffer to the column. Once you have added the Washing buffer to all columns, open the valves and let the washing buffer completely rinse through the columns.

5. Remove the Anti-EPO column from the vacuum manifold. Place it in a 1.5 mL tube and centrifuge for 1 minute at 2000 x g to remove remaining liquid in the column. Discard the tube with the flow through.

6a. Recommendation for IEF 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 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 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 the analysis requires the absence 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 as protection agent 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.

6b. Recommendation for SAR-PAGE or SDS analysis:
Place the anti-EPO column in a new microcentrifuge collection tube. Add 35-50 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. Adjustment buffer is not needed. 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!** The buffer composition in Elution buffer C 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 % casein as a protection agent. Buffer composition in Elution buffer B is the same with the following changes: Casein is replaced with 0.01% BSA. Elution buffer C is recommended, since BSA might disturb EPO Fc detection.

## 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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