# EPO Purification Kit

**Directions for Use, 101260/ 06**
Issued: Jan 2016, Revised: June 2018

## 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) and especially EPO isoforms often occur 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 techniques such as isoelectric focusing (IEF), SARCOSYL polyacrylamide gel electrophoresis (SAR-PAGE) and Membrane Assisted Isoform ImmunoAssay (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. Buffer for urine and MilliQ water are added to the urine sample to dissolve the precipitates. Exposure aid is added to enhance the interaction between pegylated EPO e.g CERA and the antibodies on the Anti-EPO column but also preventing unspecific binding to filter and surfaces. Tamm-Horsfall glycoprotein (THP) is a protein commonly found in urine which easily aggregates to macromolecules. If use of urine sample volume exceeds 10 mL, then the samples need to be heated in a boiling water bath to change the THP macromolecule structure thus prevent clogging, and thereby facilitating the flow through the narrow pores in the Anti-EPO columns. Some EPO variants or parts of the modified EPO structure e.g. EPO-Fc might be sensitive to heat. Heat treatment is not needed if urine sample volume is less than 10 mL.
Fibrinogen and high protein content in the serum/plasma samples may occasionally obstruct the pores of the Anti-EPO column and affect the flow rate through the column. This issue is not frequently found if the applied sample amount does not exceed 1 mL. Buffer for plasma or serum, Exposure aid and MilliQ water are added to the sample for the same reasons as urine sample.
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 either the use of 0.5% SARCOSYL in pH neutral buffer or an acidic buffer. EPO is then highly purified and concentrated with preserved isoform distribution in either:

- 35 µL 0.5 % SARCOSYL, 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 0.02 % NaN3, 0.1 % TWEEN 20, 0.01 % BSA which is highly recommended for analysis using SAR-PAGE.

- 55 µL 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 10 mM Glycine, 0.02 % NaN3 with adjustable TWEEN 20 (0-0.1%) and BSA (0-0.05 %). Those buffer compositions are suitable for analysis such as IEF, SAR-PAGE and MAIIA.

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 (SARCOSYL), 2 mL [a] | Ready for use | 101340 |
| | 1x Desorption buffer (Low pH), 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 mL Eppendorf micro tube, vortex, MilliQ water, microcentrifuge.

## PREPARATION OF SAMPLES

### EDTA-plasma or Serum Samples 0.5-1.0 mL

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 MilliQ water to 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 5-20 mL

Urine sample volumes that exceed 10 mL need to be heated in a boiling water bath as describe below 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.
Heat treatment is optional for urine sample volumes less than 10 mL. Clogging can however be an issue for a few urine samples, if not heated. Reduce buffers and MilliQ water by half if the sample volume is less than 10 mL.

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

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

3. Mix the urine sample gently and transfer 11-20 mL into a 50 mL polypropylene conical tube, seal the tube and boil it for **5 minutes**. Warning! The pressure in the tubes will increase as a result of the heating. Do not seal the cap tightly. Thereafter, **immediately** cool the sample in a chilled water bath for approximately 10 minutes.

4. After cooling, add MilliQ water to 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 for approximately 10 minutes.

5. 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.

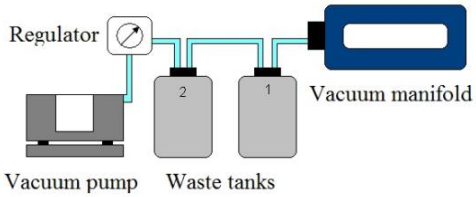


**Figure 1.** Schematic picture of a vacuum equipment set up.

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.

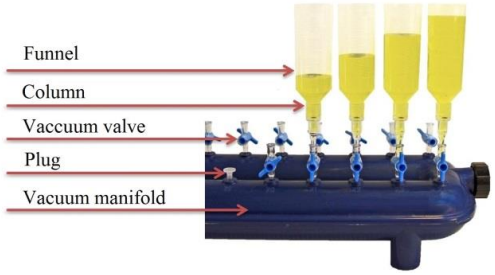


**Figure 2.** Assembling of Anti-EPO columns and funnels on vacuum manifold.

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.

Check the flow rate at 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 (Art No 101220).

3. Close the vacuum valve as soon as the sample has passed through their columns and add 1 mL Washing buffer (Art No 101280). 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 minute at 2000 x g to remove remaining liquid. Discard the tube and the waste.

5. Release bound EPO

- Option 1: Release bound EPO by the use of 0.5% SARCOSYL in pH neutral buffer. Highly recommended for SAR-PAGE.
  Place the Anti-EPO column in collecting micro tube with the same ID. Add 35 µL of Desorption buffer SARCOSYL (Art No 101340) 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. The final buffer composition will be: 35 µL 0.5 % SARCOSYL, 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 0.02 % NaN3, 0.1% TWEEN 20, 0.01 % BSA.

- Option 2: Release bound EPO by the use of an acidic buffer. Recommended for IEF and MAIIA.
  Add 5 µL Adjustment buffer into the ID marked collecting micro tube and place the Anti-EPO column with the same ID in the tube. Add 50 µL of Desorption buffer Low pH (Art No 100270) directly into the Anti-EPO column. **Immediately**, centrifuge the column for 1 minute at 2000 x g to release bound EPO.

  If use of Adjustment buffer A (Art No 100604), 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 use of Adjustment buffer B (Art No 10051), the final buffer composition will also include: 0.02 % TWEEN 20, 0.05 % BSA. A mix of Adjustment buffer A and Adjustment buffer B, (e.g. 4 µL Adjustment buffer A and 1 µL Adjustment buffer B) can also be used. At least 0.01% BSA is recommended for detection of CERA in IEF and SAR PAGE gel.

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

### APPENDIX

9 serum samples (0.5 mL) and 9 urine samples (10 mL, not heated) were spiked with 40 pg BRP, 13 pg NESP, 125 pg EPO-Fc and 200 pg CERA each. Samples were purified and eluted with 35µL Desorption buffer (SARCOSYL). All purified samples were pooled and then re-aliquoted. 1 replicate was placed in the freezer and another replicate was placed in the fridge as a constant. 5 replicates went through 1-5 freeze/thaw cycles which involved being left in room temperature for 1 h, then placed back into the freezer for 45 minutes to re-freeze. 1 blank urine and 1 serum sample was purified and placed in the freezer. Analysis was made by SAR-PAGE; single blotting, 8µL purified sample + 2µL loading buffer.

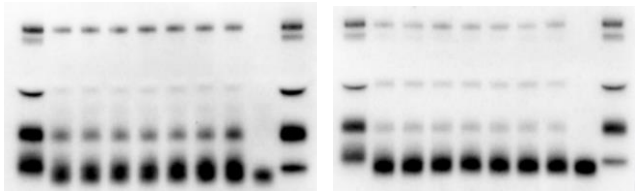

**Figure 3.** Unmodified pictures of the gels. Serum samples (left picture) and urine samples (right picture). Positions from left; ESA standard (BRP, NESP, EPO-Fc, CERA), 1 to 5 freeze/thaw cycles, -20 ˚C, + 4 ˚C, blank sample and ESA standard.

### WARRANTY



### TRADEMARKS



### 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