EPO Purification Gel Kit 3F6
For Blood

Directions for Use, 101400/ 08

Issued: Nov 2018, Revised: April 2024

INTENDED USE

EPO Purification Gel Kit 3F6 for Blood is used for rapid purification and concentration of endogenous (hEPO) or recombinant erythropoietin (rhEPO) from serum / plasma or dried blood spot (DBS) sample 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 dried blood spot (DBS), serum and plasma, erythropoietin (EPO) and especially EPO isoforms often occur at a very low concentration together with numerous other molecules. Therefore, it is often necessary to purify and concentrate EPO before analysis with techniques such as analysis with techniques such as SARCOSYL (SAR) or sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis.

PRINCIPLE OF THE PROCEDURE

Buffer is added to serum or plasma samples. After filtration the sample mixture is transferred to the disposable Anti-EPO gel column containing resin with immobilized anti-EPO antibodies 3F6 for end over end rotation. The antibody captures hEPO and rhEPO such as Epoetins, NESP, CERA and EPO-Fc. DBS samples on filter paper or other similar blood absorbing filters are treated in the same way. Buffer and the DBS sample are added in the disposable Anti-EPO gel column. EPO is extracted and purified simultaneously by end over end rotation. After purification, aqueous media is removed by pressure format. The affinity resin remaining in the column is rinsed with a washing buffer before the bound EPO is released by an elution buffer. EPO is then purified and concentrated in 0.5 % SARCOSYL, 0.1 M Bis-tris pH 7.0, 0.1 M NaCl, 0.02 % NaN3, 0.1 % TWEEN 20 with or without 0.01% casein as a protection agent. The purified sample should be stored at -20˚C until analysis.

REAGENTS

Art No	Name and Contents		
1430	EPO Purification Gel Kit 3F6 For Blood		
	Contains reagents for 25 tests.		
	Contents:		
	1x Anti EPO gel column 3F6, 25 pcs	Ready for use	101410
	1x Sample buffer, 125 mL [(a)]	Ready for use	101480
	1x Washing buffer, 175 mL[(a)]	Ready for use	101370
	1x Elution buffer, 5 mL[(a)]	Ready for use	101471
	1x Elution buffer C (incl. casein), 5 mL[(a)]	Ready for use	101560

[(a)] Contains < 0.1 % sodium azide

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 bodily 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 F20, Art No 1420

Equipment and materials required but not provided by MAIIA Diagnostics:

- Vacuum manifold with standardized Luer female taper connection and vacuum source as illustrated in Fig.3.
- 0.45 µm HPF Millex HV filter (Cat. No SLHVM25NS, Millipore) and 20 mL syringe with Luer-Lok.
- 15 mL and 50 mL conical centrifuge tube.
- Tube rotator and microcentrifuge.

PURIFICATION PROCEDURE

1. Remove the upper cap of the Anti EPO gel column 3F6 containing affinity resin and assemble the column with the Funnel F20. Place the funnel/column assembly in a 50 mL conical centrifuge tube.

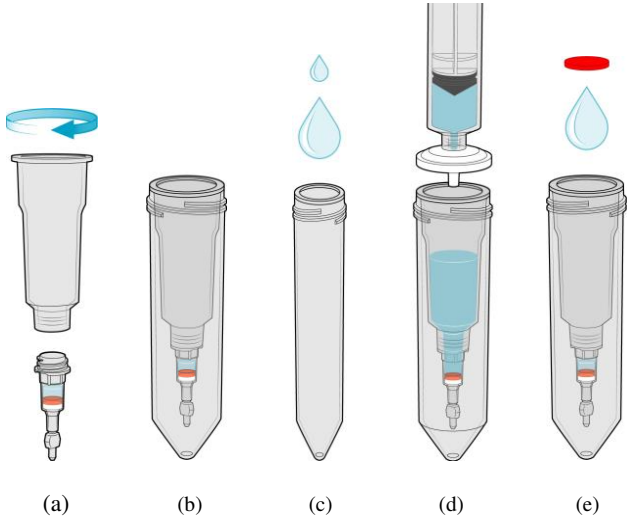
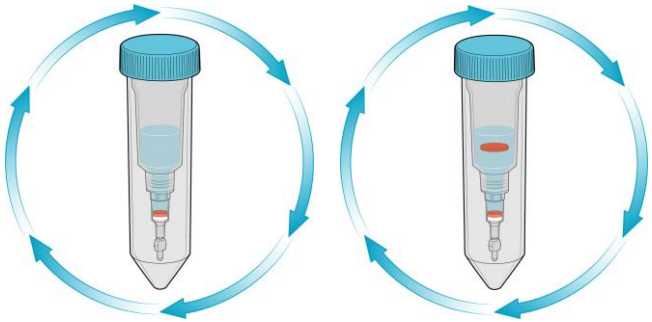


Figure 1. Anti EPO gel column assembling (a, b). Sample preparation for serum or plasma (c, d) and DBS (e) sample.

2. Add 5 mL Sample buffer and 0.2-0.5 mL serum or plasma sample into a 15 mL conical centrifuge tube and mix gently. Filter the sample mixture through the recommended 0.45 µm HPF Millex HV syringe filter and add the filtrate into the column. Prefiltering removes precipitates or cellular debris thus prevents clogging in the column and reduces background in EPO analysis. DBS filter papers or other similar blood absorbing filters and buffers can be added directly into the column. Prefiltering is not needed for DBS sample.

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 2 hours. Choose a proper rotation speed where no sample remains in the column when in the upside-down position, typically at 15-20 rpm.



(a) (b)

Figure 2. EPO purification by end over end rotation for serum or plasma sample (a) and DBS sample (b).

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 by pressure format (typically at -300 mBar) as illustrated in Figure 3.

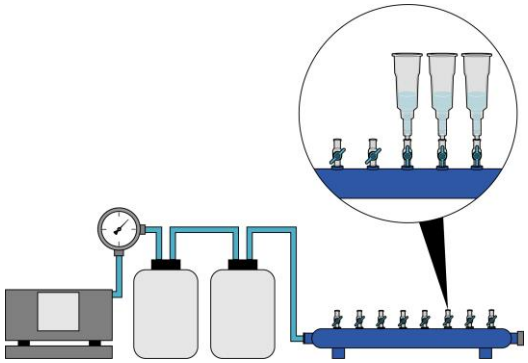


Figure 3. Schematic picture of a vacuum equipment set up. From the left: vacuum source with regulator, tanks to collect waste and vacuum manifold with standardized Luer female taper connection.

5. Close the vacuum valve as soon as the sample has passed through the column. Add 5 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.

6. Unscrew the column from the funnel. When working with DBS, make sure there is no DBS filters / blood absorbing filters in the column. Remove it if necessary. 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. Place the column in a new 1.5 mL collection tube. Add 50 µL Elution buffer or Elution buffer C into the affinity resin and elute EPO by gravity-flow for 1 minute. Then centrifuge the column with the collection tube for 1 minute at 300 x g to collect the remaining eluate.

Note: For higher yield, increase the elution buffer volume to 100 µL. Concentrate the eluate down to 15-20 µL by using a 30 kDa centrifugal cut off filter, typically spin for 45 minutes at 14 000 x g. Highly recommended for DBS samples.

Buffer composition in Elution buffer is 0.5 % Sarcosyl, 0.1 M Bis-tris, pH 7.0, 0.1 M NaCl, 0.02 % NaN3 and 0.1 % TWEEN 20. Buffer composition in Elution buffer C is the same but including 0.01 % casein as a protection agent and is recommended for long term storage.

8. Proceed with analysis or store purified samples at -20˚C until analysis. EPO might be degraded under other conditions. Discard the used Anti-EPO column.

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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