EPO WGA MAIIA Isoform Distribution Kit

Directions for Use 100790/07(EN)
Issued: March 2012, Revised: Oct 2013

INTENDED USE

EPO WGA MAIIA Isoform Distribution Kit is a combined affinity chromatography and lateral flow immunoassay for distinguishing between various recombinant human erythropoietins (rhEPO), erythropoietin analogues and endogenous erythropoietins (hEPO) in urine, serum or EDTA-plasma. It is intended for research use only.

SUMMARY AND EXPLANATION

Erythropoietin (EPO) is a glycoprotein hormone of approximately 30 kDa whose main function is control of haematopoiesis. In adults, the major site of production is the kidney but the liver also makes a significant contribution and it is also known to be produced in low amounts at other locations, such as the brain. EPO occurs in a large number of isoforms differing in their posttranslational modification (PTM) glycosylation patterns. In the case of rhEPO this gives rise to alpha, beta, delta and omega forms. hEPO as well as Exogenous EPOs can be identified in blood or urine due to the above mentioned differences in their isoform patterns.

PRINCIPLE OF THE PROCEDURE

The EPO WGA MAIIA (Membrane Assisted Isoform ImmunoAssay) method combines a wheat germ agglutinin (WGA) affinity chromatography with a consecutive immunochemical detection on a lateral flow membrane. A schematic picture of the strip are shown in figure 1.

Before running the EPO WGA MAIIA method, EPO from serum, EDTA-plasma or urine sample needs to be affinity purified and concentrated using the EPO Purification Kit from MAIIA Diagnostics. When purified EPO is applied on the EPO WGA MAIIA strips, all EPO isoforms are first retained to the immobilized WGA ligands in the separation zone. Bound EPO is then released by a WGA competing sugar derivative, N-acetylglucosamine (GlcNAc). The WGA ligands interact differently with different isoforms of EPO. To allow different isoform profiles to be distinguish, all samples are analyzed using two or more elution buffers: on one strip an elution buffer with a high concentration of GlcNAc is used, resulting in migration of all isoforms and a determination of the total amount of EPO. On the other strip an elution buffer with a lower concentration of GlcNAc is used, which will result in migration of certain isoforms only. Released EPO from both strips is captured downstream on the detection zone with immobilized anti-EPO antibodies and visualized with Anti-EPO Carbon Black Nano-Strings (Anti-EPO CBNS). This sandwich configuration yields a black to grey signal quantified with a flatbed scanner. The blackness intensity is converted into values for EPO concentration determination by the image processing software MAIIAcalc.

Percentage of Migrated Isoforms (PMI) is the ratio of the concentration values for the strips eluated with low and high concentration of GlcNAc respectively. PMI can be used to distinguish samples with different isoform profiles. See Figure 2. More comprehensive information can be found in reference[1].

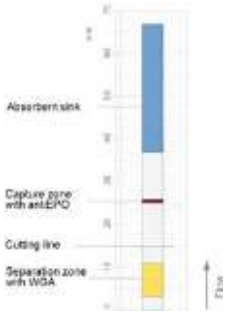

Figure 1. EPO WGA MAIIA strip with separation and detection zone.

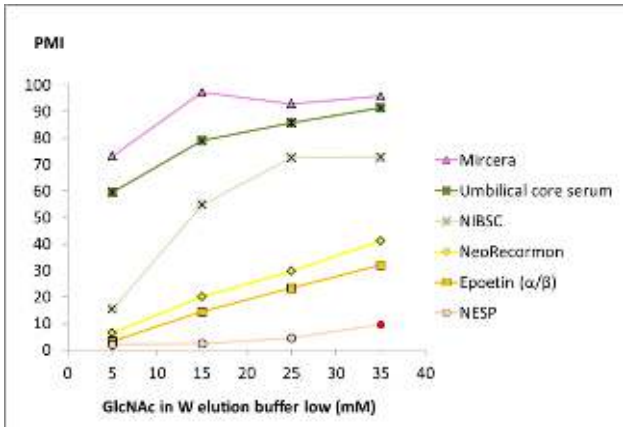

Figure 2. PMI values for NESP, Epoetin (α/β), NeoRecormon, NIBSC, EPO from umbilical cord serum and Mircera when using different GlcNAc concentrations in W elution buffer *low*.

REAGENTS

Art. No.	Name and Contents		
1310	EPO WGA MAIIA Isoform Distribution Kit		
	Contains 150 strips.		
	Contents:		
	3x EPO WGA MAIIA strips, 50 pcs	Ready for use	100770
	1x Epoetin (α/β), 10 µg/L, 0.45 mL [(a)]	Stock solution	100910
	1x Prewashing buffer, 5 mL [(a)]	Ready for use	100730
	1x Sample dilution buffer, 20 mL [(a)]	Ready for use	100233
	1x W elution buffer high, 5 mL [(a)]	Ready for use	100780
	1x Anti-EPO CBNS, 3 mL [(a)]	Stock solution	100064
	1x CBNS dilution buffer, 3 mL [(a)]	Stock solution	100075
	1x Washing buffer, 10 mL [(a)]	Ready for use	100084
	1x Scanning template, 6 pcs		100192

[(a)] Contains <0.1% sodium azide

Storage and Shelf Life

Store all components at + 4-8°C. Do not freeze kit 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 fluids must be handled and treated as a potentially infectious.
- 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 Required

Materials required and available from MAIIA Diagnostics:

- EPO Purification Kit, Art No 1360
- Funnel Pack F40, Art No 1340
- EPO WGA MAIIA Software, Art No 1351

Materials required but not provided by MAIIA Diagnostics:

- Flat bottomed Microtiter wells (e.g. Nunc PolySorp™, Art No 469957), Nunc Frame (Art No 460348), forceps, electronic multipette dispensers, scissors, transparent tape
- Epson Perfection® V700 Photo Scanner or Epson Perfection V750-M Pro Scanner and scanner software SilverFast AiStatistical software capable of generating a four parameter logistic curvefit

PROCEDURE

The system calibration below should be checked for each new lot and then once a month.

For EPO concentration determination of unknown samples a calibration solution and an immunoassay curve-fitting programme with four parameter logistic curve-fit is required. To distinguish various EPO types or populations with best possible resolution, the W elution buffer *low* must first be optimized. To differentiate between, e.g. NIBSC and NeoRecormon, W elution buffer *low* at 15-25 mM GlcNAc range is recommended. PMI value for epoetin (α/β) in this case is about 20. But if the objective is to discriminate between endogenous kidney EPO and Mircera or EPO from umbilical cord serum, W elution buffer *low* at 5 mM GlcNAc would be more suitable. PMI value for epoetin (α/β) in this case is about 5 (Figure 2).

System Calibration

Testing conditions:
Temperature +20-25°C
Humidity 10-80 RH%

1. Bring all reagents to room temperature before use.

2. Prepare EPO calibration solutions of 0, 10, 30, 100, 300, 600 and 1000 ng/L as shown in Table 1.

Table 1. Preparation of calibration solutions. SDB = Sample dilution buffer (Art No 100233). EPO stock = Epoetin (α/β) (Art No 100910).

Typical delta blackness/pixel	EPO solutions	
	1000 ng/L	540 µL SDB + 60 µL EPO stock
24281	600 ng/L	200 µL SDB + 300 µL 1000 ng/L
18729	300 ng/L	350 µL SDB + 150 µL 1000 ng/L
7757	100 ng/L	450 µL SDB + 50 µL 1000 ng/L
2930	30 ng/L	450 µL SDB + 50 µL 1000 ng/L
1454	10 ng/L	450 µL SDB + 50 µL 100 ng/L
622	0 ng/L	500 µL SDB

3. Prepare EPO sample. 300 µL 300 ng/L EPO is needed. Dilute epoetin (α/β) stock solution with Sample dilution buffer. Preferably, also make dilutions of two samples representative of those to be separated and add those to the procedure below.

4. Prepare W elution buffer *low* in 5, 15, 25, 35 and 50 mM GlcNAc as shown in Table 2.

Table 2. Preparation of W elution buffer low solutions.

W elution buffer low	
50 mM GlcNAc	375 µL SDB + 75 µL W elution buffer high
35 mM GlcNAc	60 µL SDB + 140µL 50 mM GlcNAc
25 mM GlcNAc	100 µL SDB + 100 µL 50 mM GlcNAc
15 mM GlcNAc	140 µL SDB + 60 µL50 mM GlcNAc
5 mM GlcNAc	180 µL SDB + 20 µL50 mM GlcNAc

5. Prepare Anti-EPO CBNS working solution by mixing 1 part of Anti-EPO CBNS (Art No 100064) with 1 part of CBNS dilution buffer (Art No 100075). Vortex each reagent before mixing! 600 µL Anti-EPO CBNS working solution is needed, plus 250 µL extra for each added sample to be included in the optimization of GlcNAc concentration. Use within the same day. Discard after use.

6. Mark all strips and place the strips on a clean area. 22 x EPO WGA MAIIA strips (Art No 100770) are required. Add 10 extra strips for each extra sample included.

7. Place microtiter wells into Nunc Frames. Dispense Prewashing buffer, sample mixture, W elution buffer low / high, Anti-EPO CBNS working solution and Washing buffer at the forefront of the wells with volumes of 25 µL per well. See Figure 3.

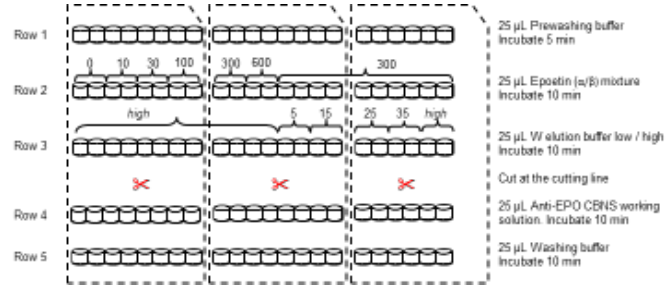

Figure 3. Setup when running the calibration curve and optimization of W elution buffer *low*.

8. Immerse the thin end of the strips one by one into the wells of row 1 with 5 seconds interval between each strip. Move the strips in the same order and time interval to the next row according to the incubation schedule and cutting shown in Figure 3. Do not interrupt the process until row 5.

9. Remove the protective slip on the Scanning template (Art No 100192) and mount the strips with the plastic backing facing down on the scanning template. The strips must be within the white boxes and covering the dot. Incorrect montage may cause failure of signal detection by the scanner software. See Figure 4.

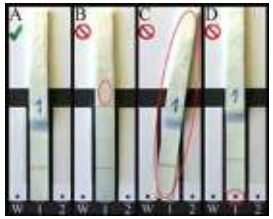

Figure 4. (A) Correct montage. The strips within the white boxes with the colour code facing up. (B) Incorrect montage. Strips with the plastic backing facing up. (C) Incorrect montage. The strip is mounted obliquely. (D) Incorrect montage. The strip is mounted not covering the dot.

10. Remove the absorbent pads from the strips using forceps. Cover the unused adhesive part with transparent tape. Let the strips dry at room temperature in a draft-free environment for 1 hour or more.

Scanning Detection and Calibration of W elution buffer *low*

For a full description of the scanning procedure and the use of MAIIAcalc please see the DfU Scanner Detection Instruction (Art No 100530, MAIIA Diagnostics).

1. Open the Calculation of PMI values.xls file. To calculate the optimal dilution of the W elution buffer *low,* open the "Opt. of W elution buffer low"–sheet.

2. Enter the parameters (a-d and base) for the calibration curve in the assigned cells. The calibration curve parameters are obtained when generating a four parameter logistic curve-fit of the delta blackness/pixel (dbl/pix) values for the standard curve derived by MAIIAcalc. Base 1 and 2 values are the dbl/pix of point 0 ng/L of the standard curve.

3. Enter the delta blackness/pixel values obtained from MAIIAcalc for the W elution buffers *low* solutions 5, 15, 25 and 35 mM W elution buffer *high* solution into the assigned cells. The output data are EPO concentrations for elution *low* (ng/L) and elution *high* (ng/L), imprecision of the duplicates (CV %) and PMI value.

4. Identify the suitable W elution buffer *low* to be used in the assay, by comparing the results with the epoetin (α/β) graph in Figure 2.

Sample Preparation

EPO isoforms normally occur at very low concentrations together with numerous other molecules in urine, serum and plasma. It is necessary to purify and concentrate EPO with the EPO Purification Kit (Art No 1360) before isoform determination with the EPO WGA MAIIA method. Recommended sample volumes are 20 mL urine, 1-2 mL serum or 1-2 mL EDTA-plasma. After purification, EPO will be obtained in 55 µL eluated sample with preserved isoform distribution.

Sample Test Procedure

Testing conditions:
Temperature +20-25°C
Humidity 10-80 RH%

1. Bring all reagents and affinity purified samples to room temperature.

2. Prepare a sample list. A maximum of 15 samples including controls can be run per assay. It is recommended to always include the epoetin (α/β) provided with the kit as a control, diluted to 300 ng/L.

3. EPO needed for running the EPO WGA MAIIA method in duplicates is 100 µL per sample, within 50-500 ng/L range after dilution. Prepare sample, e.g. add 10 µL affinity purified sample to 100 µL Sample dilution buffer (Art No 100233) and vortex.
NOTE! *The affinity purified sample may have to be diluted more or less than we have recommended, since the EPO concentration in biological fluids is highly variable, especially in urine samples.*

4. Prepare Anti-EPO CBNS working solution by mixing 1 part of Anti-EPO CBNS (Art No 100064) with 1 part of CBNS dilution buffer (Art No 100075). Vortex each reagent before mixing! 100 µL Anti-EPO CBNS working solution is needed per sample. Use within the same day. Discard after use.

5. Prepare a suitable W elution buffer *low* obtained from the System Calibration procedure. 50 µL of each of W elution buffer *high* and *low* is needed per sample.

6. Mark all strips and place the strips on a clean area. 4 x EPO WGA MAIIA strips (Art No 100770) are required for per sample.

7. Place microtiter wells into Nunc Frames. Dispense Prewashing buffer, sample mixture, W elution buffer low and high, Anti-EPO CBNS working solution and Washing buffer at the forefront of the wells with volumes as shown in Figure 5.

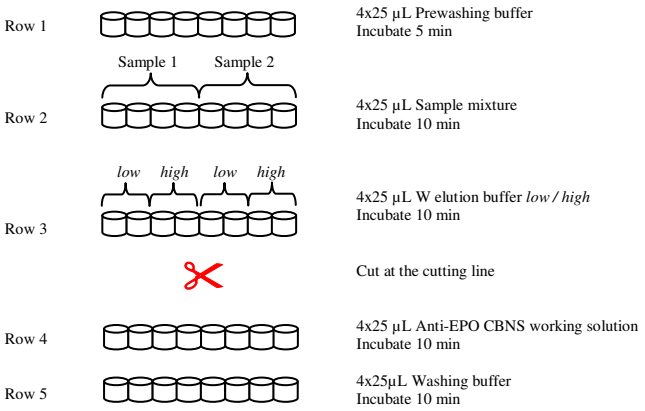

Figure 5. Sample test setup for 2 samples. Volume for 1 sample.

8. Immerse the thin end of the strips one by one into the wells of row 1 with 5 seconds interval between each strip. Move the strips in the same order and time interval to the next row according to the incubation schedule and cutting shown in Figure 5. Do not interrupt the process until row 5.

9. Remove the protective slip on the Scanning template (Art No 100192) and mount the strips with the plastic backing facing down on the scanning template. The strips must be within the white boxes and covering the dot. Incorrect montage may cause failure of signal detection by the scanner software.

10. Remove the absorbent pads from the strips using forceps. Cover the unused adhesive part with transparent tape. Let the strips dry at room temperature in a draft-free environment for 1 hour or more.

Scanning Detection and Calculation of Percentage of Migrated Isoforms

For a full description of the scanning procedure and the use of MAIIAcalc please see the DfU Scanner Detection Instruction (Art No 100530, MAIIA Diagnostics).

1. Open the Calculation of PMI values.xls file. Open the "EPO WGA MAIIA Method" sheet for calculating PMI values.

2. Enter the parameters (a-d and base) for the standard curve in the assigned cells. The standard curve parameters were obtained from the System Calibration procedure.

3. Enter the delta blackness/pixel values obtained from MAIIAcalc for the samples into the assigned cells. The output data are EPO concentrations for elution *low* (ng/L) and elution *high* (ng/L), imprecision of the duplicates (CV %), PMI value and EPO concentrations for the samples if their dilution factors are entered into the assigned cells.
NOTE! *The EPO concentration should be within a range of 10-500 ng/L to get an accurate PMI value. If not, re-run sample with another dilution factor.*

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by MAIIA AB 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 authorized distributors, in such event, shall not be liable for damages indirect or consequential.

REFERENCES

[1]Lönnberg, M., Andrén, M., Birgegård, G., Drevin, M., Garle, M., Carlsson, J. Analytical Biochemistry 420(2), 101-114 (2012) Rapid detection of erythropoiesis-stimulating agents in urine and serum.

TRADEMARKS

MANUFACTURER

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