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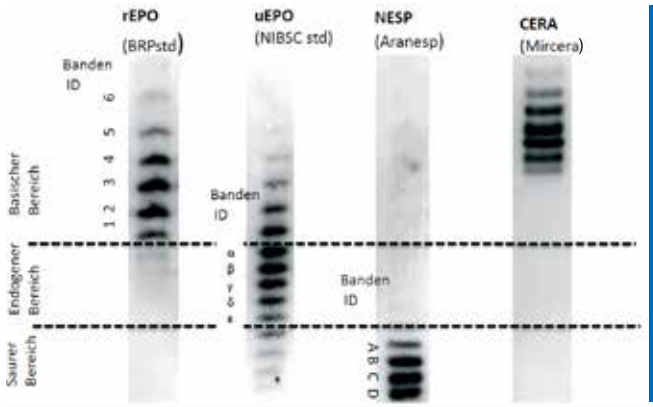
Specials

# EPO Doping IEF Kit 30S

## A Precast IEF Gel Kit for Use in EPO Doping Control

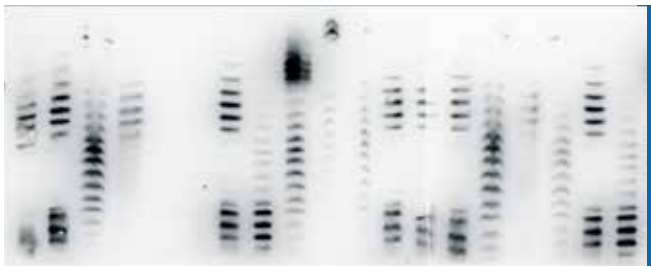
### Why isoelectric focusing in EPO doping control?

Natural erythropoietin (EPO) differs from recombinant EPO by the length of the carbohydrate chains, whereas the amino acid sequence is identical. The difference in glycosilation leads to different isoelectric points of the isoforms, and therefore in IEF to distinct bands according to the pI of the individual protein species. After isoelectric focusing the separated protein bands are blotted onto a membrane by pressure blotting. Secure verification of EPO isoforms is then done by immune-detection.




IEF according to WADA procdures. With courtesy of Institute of Biochemistry, German Sport University Cologne. Accredited WADA laboratory.

- Precast gels – no gel preparation
- Long shelf life of gels
- Everything for IEF provided with the kit
- Easy, standardized procedure
- Sample loading into 30 slots per gel
- Suited for press blotting



IEF results of EPO standards using SERVA EPO Doping gel kit. With courtesy of Institute of Doping Analysis and Sports Biochemistry, Kreischa. Accredited WADA laboratory.

### The EPO Doping Kit

The EPO Doping IEF Kit 30S is especially developed for EPO IEF analysis, precast polyacrylamide gels are included. Therefore there is no need of tediously casting of IEF flatbed gels on support films. As urea will degrade the acrylamide matrix the CleanGel EPO IEF 30S gels are manufactured by SERVA as dry acrylamide gels without urea and carrier ampholytes. These gels are stable at – 20 °C for at least two years. Before use gels have to be rehydrated by a simple one step procedure. The urea and SERVALYT™-containing rehydration solution is made from rehydration additive and the SERVALYT™ EPO Mix, both supplied with the kit. Now the gel is ready for horizontal IEF of urine protein samples in EPO doping control.

### Background information EPO doping control

According to the „Summary of Major Modifications“ of the document TD2014EPO, renamed into “Harmonization of Analysis and Reporting of Erythropoiesis-Stimulating Agents (ESAs) by Electrophoretic Techniques” (published by WADA), IEF and/or SAR-PAGE should be applied for the initial testing procedure of ESAs with a chemical structure related to EPO (rEPO, NESP, CERA, EPO-Fc). For peginesatide, a pegylated peptide with no structural relationship to EPO, laboratories shall apply SDS-PAGE or SAR-PAGE. The presence of rEPO shall be confirmed by SDS-PAGE or SAR-PAGE while for the confirmation of CERA IEF or SAR-PAGE should be used. Precast gels for SAR-PAGE will be released by SERVA shortly.

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## Equipment

To run the EPO gels a standard flatbed chamber with cooling system (8 °C) and a power supply (2000 V, 50 mA, 30 W) are needed. For optimal and consistent results SERVA recommends the usage of the new SERVA HPE™ systems. One option is the HPE™ BlueHorizon™, which is a single platform system that could be combined to a double, triple or quadra deck. Another option for high-throughput applications in IEF is the HPE™ BlueTower System allowing electrophoretical separations of up to four horizontal gels at the same time.


The HPE™ BlueHorizon™ is made from the same components as the HPE™ BlueTower but designed as a single flatbed system for optimal performance in cooled horizontal isoelectric focusing applications. Up to 4 units are stackable.


The HPE™ BlueTower is a multilevel flatbed electrophoresis device providing unmatched resolution and reproducibility in horizontal IEF.

## The Protocol

### I. Gel rehydration

Mix 23.5 g rehydration additive with 6 ml SERVALYT™ EPO Mix in a final volume of 50 ml dd$H_2O$ and transfer the solution into a rehydration tray. Place the gel with gel side down on the surface of the solution without trapping any air bubbles and incubate for 2 h (optional over night). Dry the gel surface with the drying cardboard.

### II. Electrophoresis

Prepare anode and cathode buffer and wet the electrode wicks on an absorbent sheet. Cool the IEF unit to 8 °C. Pipet 2.5 ml cooling contact fluid (SERVA cat. no. 43371) on the cooling plate. Place the gel air bubble-free on the cooling plate, sample slots on the cathodic side of the gel. Finally place the wetted cathode and anode electrode wicks on the gel. Start electrophoresis under the following focusing conditions:

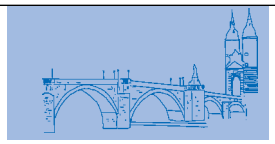
1. Pre-focusing: 250 V, 30 mA, 30 W for 30 min
2. Sample application
3. Focusing: 2.000 V, 50 mA, 30 W for 4.000 Vh.

After electrophoresis is complete following your blotting and immune-detection protocol.

## Ordering Information

| Product | Quantity | Cat. No. |
|---|---|---|
| EPO Doping IEF Kit 30S | 1 kit | 43389.01 |
| HPE™ BlueHorizon™ System | 1 unit | HPE-BHS |
| HPE™ BlueTower System | 1 unit | HPE-TS2 |

Each EPO Doping IEF Kit 30S contains 4 CleanGels EPO IEF 30S, Rehydration Additive (4 x 23,5 g), SERVALYT™ EPO Mix (4 x 6 ml for rehydration) and SERVALYT™ 6 – 8 (1 x 2 ml, for making cathode buffer), drying cardboards (4 pcs.) and electrode wicks (8 pcs.).



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