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IVD

CE

MediRox

# Instructions for Use [EN]

## MRX Blue Free Protein S

REF K5010

For *In vitro* Diagnostic Use.

### 1 Intended use

Latex immunoassay for quantitative determination of Free Protein S (FPS) in citrated human plasma as an aid in diagnosis of Protein S deficiency. Intended to be used by professional laboratory personnel using coagulation analysers with turbidimetric detection in the 400 - 600 nm wavelength range.

### 2 Background and principle of method

Protein S is a vitamin K dependent glycoprotein with anticoagulant properties.[1,2,3,4] In the presence of calcium, Protein S acts as a cofactor to, and forms a complex with, Activated Protein C (APC). The complex adheres to negatively charged phospholipid membranes which increases the anticoagulant activity of APC.[4,5] Protein S deficiencies, both congenital and acquired, are associated with increased risks for deep vein thrombosis (DVT) and pulmonary embolism (PE).[6,7]

The normal total concentration of Protein S in human plasma is 20 – 25 mg/L. Approximately two thirds of this is bound to C4 Binding Protein (C4BP), while the remaining non-bound fraction is free Protein S.[2,8]

MRX Blue Free Protein S consists of Protein S specific monoclonal antibodies coupled to sub-micron sized polystyrene particles. MRX Blue Free Protein S is only sensitive to free Protein S and will not react with protein S bound to C4BP. When the reagent is exposed to a plasma sample containing free Protein S, the particles will agglutinate, giving rise to increased light-scattering. When exposed to the appropriate wavelength of light, the increase in measured turbidity, or light-scattering, is proportional to the amount of FPS in the sample.

### 3 Components

MRX Blue Free Protein S consists of:

- Latex Reagent: 3 × 2.5 mL polystyrene particles, coated with monoclonal antibodies, suspended in buffer with stabilisers (porcine origin) and preservatives.
- Reaction Buffer: 3 × 4 mL containing buffer, Heterophilic Blocking Reagent (HBR), and preservatives.

### 4 Warnings and precautions

Wear suitable clothing for protection. Avoid contact with skin and eyes. Do not empty into drains. Waste must be disposed of in accordance with local regulations.

The Latex Reagent and Reaction Buffer contain sodium azide (less than 0.1%) and 2-methylisothiazol-3(2H)-one (less than 0.0015%) to prevent microbial growth; use proper disposal procedures.

EUH208: Contains 2-methylisothiazol-3(2H)-one. May produce an allergic reaction.

EUH210: Safety data sheet available on request.

### 5 Preparation

- Latex Reagent: Ready to use. As the microparticles will settle during storage, swirl the vial gently a few times every day before use to ensure a homogenous suspension. Do not shake.
- Reaction Buffer: Ready to use. Swirl the vial gently a few times before use.

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## 6 Storage and stability

- Latex Reagent: Store at 2 - 8 °C. Do not freeze. After opening, stable for 8 weeks at 2 - 8 °C in the closed original vial, provided no contamination occurs. On-board stability: 7 days on Sysmex CS-2100i.
- Reaction Buffer: Store at 2 - 8 °C. Do not freeze. After opening, stable for 8 weeks at 2 - 8 °C in the closed original vial, provided no contamination occurs. On-board stability: 7 days on Sysmex CS-2100i.

## 7 Specimen collection and preparation

Venous blood is collected in 3.2% sodium citrate at a ratio of 9 parts blood to 1 part anticoagulant (1:10 ratio). The ratio is critical. Trauma or stasis during blood sampling should be avoided. Inverse immediately after sampling. The presence of any clots in a specimen is a cause for rejection. Centrifuge to produce platelet-poor plasma and use for analysis. Refer to CLSI guideline H21-A5 for further instructions on specimen collection, handling and storage.[9]

## 8 Procedure

For each instrument, refer to its operator's manual and to the instrument-specific application sheet.

## 9 Material required but not provided

Coagulation analyser capable of turbidimetric detection in the 400 - 600 nm wavelength range, pipettes and the following:

| Calibrator | REF |
|---|---|
| MRX Free Protein S Calibrator | K5044 |

| Control material | REF |
|---|---|
| MRX Specialty Normal Control | K5021 |
| MRX Specialty Abnormal Control | K5022 |

| Solutions | REF |
|---|---|
| Phosphate buffered saline (PBS) for dilution, e.g. MRX PBS Diluent | K5047 |
| Deionised water for reconstitution e.g. MRX Laboratory Water | K5036 |

## 10 Quality control

To maintain consistent assay results, it is recommended that control plasmas are assayed at regular intervals. MRX Specialty Controls (K5021/K5022) are recommended for MRX Blue Free Protein S. Each laboratory should establish a control range to determine the allowable variation in the day-to-day performance of the test, as well as appropriate intervals for analysing controls in accordance with good laboratory practice. Recalibration is suggested, as a minimum, whenever control plasmas are not within the acceptable range and each time a new batch of reagent is used.

## 11 Results

The results are reported in % FPS, where 100% corresponds to 1 IU/mL FPS of the WHO 2nd International Standard (NIBSC code 03/228).

Samples that are reported above the measuring range should be manually diluted and re-analysed. No result outside the measuring range should be used in forming a diagnosis or for patient management.

## 12 Expected values

Normal Free Protein S levels for females are typically in the 54 - 155% range, while levels for males are somewhat higher in the 68 - 176% range.[10]

Results from FPS concentrations determination in plasma samples from 120 healthy blood donors using MRX Blue Free Protein S are presented below. The analysis was performed using a Sysmex CS-2100i instrument.

| No of samples | Mean FPS | 95% reference interval |
|---|---|---|
| 120 | 102% | 39 - 165% FPS |

Due to inter-laboratory variation, each laboratory should set its own reference interval.

## 13 Limitations and interfering substances

The results should be used together with other clinical and diagnostic information in forming a diagnosis and for patient management.

During pregnancy, the level of Free Protein S in plasma may be altered and, consequently, there is a risk of incorrect results when analysing free Protein S on samples from pregnant women. The recommendation from the International Society of Thrombosis and Haemostasis

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(ISTH) is, therefore, to not analyse free Protein S during pregnancy.[11] If free Protein S is analysed during pregnancy, despite the recommendations not to, the analysis must be repeated postpartum. With regards to the ISTH recommendations, MRX Blue Free Protein S should not be used to analyse free Protein S on samples from pregnant women.

Turbid or opalescent plasma may cause erratic results and should be interpreted with caution: dilute the sample and re-assay. MRX Blue Free Protein S is insensitive to the following substances on Sysmex CS-instrument series:

| Interfering substance | Tolerance |
|---|---|
| Bilirubin | Up to 18 mg/dL |
| Haemoglobin | Up to 500 mg/dL |
| Triglycerides | Up to 230 mg/dL |
| Unfractionated heparin | Up to 330 U/dL |
| Low molecular weight heparin | Up to 330 U/dL |

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain anti-mouse antibodies (HAMA), which may cause over-estimation of free Protein S concentrations. The presence of rheumatoid factor may also result in falsely elevated free Protein S concentrations. The reaction buffer includes HBR that reduces unspecific reactions, but users should be aware that there still is a possibility of over-estimated free Protein S levels for samples with HAMA or rheumatoid factor. MRX Blue Free Protein S is insensitive to 1000 IU/mL of rheumatoid factor and 1280 x HAMA.

The monoclonal antibodies in MRX Blue Free Protein S have been screened for their specificity for free Protein S. When purified C4BP is added to normal plasma samples, in amounts that are stoichiometrically equivalent to the normal FPS plasma concentration, the signal reported by MRX Blue Free Protein S is fully quenched.

## 14 Analytical performance characteristics

The following performance data was obtained with a Sysmex CS-2100i instrument. Performance will depend on the instrument used.

MRX Blue Free Protein S has a measuring range of 7 - 200% FPS. When compared to another microparticle enhanced immunoassay, MRX Blue Free Protein S correlates as follows:

y (Siemens INNOVANCE free PS Ag on Sysmex CS-2100i) = 0.94 x (MRX Blue Free Protein S on Sysmex CS-2100i); $r^2 = 0.99$.

Precision:

| Sample | Mean FPS | Repeatability CV |
|---|---|---|
| Level 1 | 28% | 2.9% |
| Level 2 | 86% | 2.5% |

## 15 Reporting of incidents

Any serious incidents that occur in relation to this device shall be reported to Nordic Biomarker as well as the national competent authority in which the user is established.

## 16 Additional information

A paper copy of these Instructions for Use is available on request. Contact your local distributor.
The instrument-specific application sheet is available from your local distributor.

## 17 References

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8. REZENDE, Suely Meireles; SIMMONDS, Rachel Elizabeth; LANE, David Anthony. Coagulation, inflammation, and apoptosis: different roles for protein S and the protein S–C4b binding protein complex. *Blood*, 2004, 103.4: 1192-1201.

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10. C. DYKES, Anne, et al. A study of Protein S antigen levels in 3788 healthy volunteers: influence of age, sex and hormone use, and estimate for prevalence of deficiency state. *British journal of haematology*, 2001, 113.3: 636-641.
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## 18 Definition of symbols

| | | | |
|---|---|---|---|
|  | Manufacturer |  nordicbiomarker.com/IFU | Consult electronic instructions for use |
|  | CE mark |  | Use-by date |
|  | In vitro diagnostic medical device |  | Temperature limit |
|  | Catalogue number |  | Contains biological material of animal origin |
|  | Batch code | | |

## 19 Revision history

| Version | Changes to previous version |
|---|---|
| 7.0 | New IFU design. As a result, all sections have been updated. |



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