



## DETERMINATION OF PROTEIN FRACTIONS OF HUMAN BLOOD SERUM

### INTRODUCTION

The method allows determination of relative amounts of albumin and globulins of the human blood serum using capillary electrophoresis methods.

### MEASURING METHOD

Determination of albumin and globulins of the human blood serum is based on their migration and separation in the electric field due to different electrophoretic mobility. Identification and quantitative determination of the analysed proteins is performed by direct detection by measuring the UV absorption in 215–220 nm region.



### REFERENCE VALUES OF PROTEIN FRACTIONS DISTRIBUTION IN BLOOD SERUM

Reference values of protein fraction distribution in blood serum as a percentage of the total content are listed in the table below.

Fraction	Percent of total protein content, %
$\gamma$ -globulin ( $\gamma$ -GI)	10.7–19.2
$\beta$ -globulin ( $\beta$ -GI)	8.6–13.7
$\alpha_2$ -globulin ( $\alpha_2$ -GI)	5.2–10.7
$\alpha_1$ -globulin ( $\alpha_1$ -GI)	3.7–7.8
Albumin (Alb)	54.7–68.7

### ADVANTAGES OF CAPILLARY ELECTROPHORESIS METHOD

Compared with other methods for determination of proteins in blood serum using electrophoresis and acetate cellulose and agarose gels, capillary electrophoresis has several advantages:

- No special sample preparation
- Real-time detection
- Easy to make
- Quantitative determination
- Low analysis cost

### EQUIPMENT AND REAGENTS

The following equipment and reagents are used in measurements:

- The CAPEL<sup>®</sup>-105 Capillary Electrophoresis System
- Distilled water
- Albumin
- Sodium hydroxide, Ultra Pure Grade
- Sulfuric acid, Ultra Pure Grade
- Sodium tetraborate, Ultra Pure Grade
- Sodium dodecyl sulphate (SDS), Ultra Pure Grade



Data acquisition, collection, processing and output are performed using a personal computer running under WINDOWS<sup>®</sup> 98/ME/NT/2000/XP operating system with installed Chrom&Spec<sup>®</sup> software package for acquisition and processing of chromatography data.

#### PREOPERATIONAL PROCEDURES

Pre-operational procedures include sampling and sample preparation, capillary conditioning, preparation of auxiliary and calibration solutions, and calibration of the CAPEL<sup>®</sup> Capillary Electrophoresis System.

#### MEASUREMENT PROCEDURE

##### **Sample collection**

Collection of the sample (venous blood) must be made in accordance with the clinical requirements. The blood serum is obtained using standard protocols.

##### **Sample preparation**

Before the analysis, the blood serum should be diluted with distilled water (dilution ratio 50:1), stirred thoroughly and centrifuged.

##### **Capillary conditioning**

The capillary is rinsed sequentially for 10 minutes each time by solutions of concentrated sulfuric acid, distilled water, sodium hydroxide (1 mol/l), distilled water, and buffer solution.

##### **Measurement**

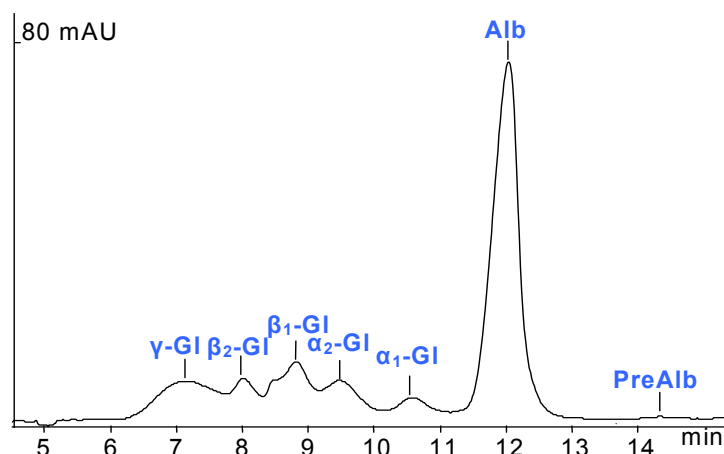
The prepared solution is analyzed by capillary electrophoresis method using the routine preset in the CAPEL<sup>®</sup>-105 capillary electrophoresis system.

#### DATA PROCESSING

Chrom&Spec<sup>®</sup> software reports the albumin and globulins content (in %).

#### EXAMPLE OF REAL ANALYSIS

**Sample:** human blood serum (normal)  
**Buffer:** 10 mM borate, 5 mM SDS, pH 9.2  
**Capillary:**  $L_{\text{eff}}/L_{\text{tot}} = 50/60$  cm, ID= 75  $\mu\text{m}$   
**Injection:** 150 mbar\*sec  
**Voltage:** 15 kV  
**Detection:** direct, 215 nm



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