Serum Proteins Electrophoresis Procedure

SELEO TYPE INSTRUMENTS KIT **REF** 08CSEL-SP

Intended use

The Serum Protein Electrophoresis (SPE) kit is intended for the separation of proteins in human serum by electrophoresis on cellulose acetate strips. Human serum proteins are separated into five zones or bands which are composed of many individuals proteins. The patterns are examined visually for abnormalities. The kit is used with the automated instruments **SELEO type**.

Summary

Human body fluids contains a varied mixture of proteins and protein complexes. Each of these protein entities apparently fulfills a specific function within the life process; furthermore, it is well known that the levels of various proteins in blood serum bear a close relationship to states of health and disease. In fact, the concentration and compositions of the over one hundred proteins contained in the serum may vary due to physiological conditions. Electrophoresis is a well established and versatile technique, routinely used in clinical laboratories. Serum protein electrophoresis performed at pH 8.8 yields five bands: Albumin and four globulins (alpha 1, alpha 2, beta and gamma). About sixteen of the known proteins contribute to the formation of the five bands in the electrophoretic pattern. Evaluation of single bands by visual inspection provides valuable diagnostic support as it offers a display of the major proteins involved in functional and pathological processes.

Principle

Electrophoresis separates serum proteins based on the premise that the individual protein species have different mobilities when subjected to an electric field. Every molecule possesses an electrical charge due to the presence of positively charged groups and negatively charged groups. The net charge dictates the migration characteristics of the species at a given pH. With the **SELEO type** Serum protein procedure, proteins are separated at an alkaline pH using the principle of Zone Electrophoresis on a suitable support medium: cellulose acetate. When the migration is complete, the proteins are stained and fixed with Ponceau red solution and then washed with a specific destaining solution.

Warning: This kit is for in vitro diagnostic use only.

Specimen Collection and Handling

Serum samples should be collected using the laboratory's procedures and in accordance with Good Laboratory Practice (GLP) Guidelines. Fresh serum samples without hemolysis or lipemia are the optimal choice for testing. Due to interference of fibrinogen, plasma is not recommended. Serum samples may be stored covered at 15° to 30° C for 4 days or 2° to 6°C for two weeks, or -20°C for 6 months.

Reagents

Reagents are supplied in concentrated solutions and ready to use. Please reference the operator's manual for the correct addition of reagents to the Instrument reagent rack.

Storage and stability

Store all reagents at room temperature (+15° to +25°C). All reagents are stable until the expiration date indicated on the label.

Strips

Cellulose acetate supported on Mylar®

Buffer (concentrated solution)

Contains: <10% Tris Base

Staining Solution ready to use

Contains: Ponceau S Red, <5% Acetic acid

Destaining (concentrated solution)

Contains: Citric Acid <50%

Warning: Irritant for eyes and skin

Mylar® is a trademark of DuPont company.

Items Provided

Kit product number: **08CSEL-SP**
For **SELEO type** instrument 400 tests

REF	DESCRIPTION	Q.TY
01H12-25	Strips - Dry Plates pack of 25	2
02C13-6NA	Buffer 100 mL	1
03C02-S	Staining Solution 200 mL	2
04A02-C20	Destaining Solution 250 mL	3
	Blotting Paper Set	1

Reagents and Materials Required But Not Provided

- Serological pipettes. Preferably pipetting devices 10-100µL
- Distilled Water
- Serum Protein Control

Test Procedure

Migration Chamber and Reagents

Insert Blotters in the slots of the migration chamber. Complete the preparation of Reagents as indicated on the Operator's Manual. Load diluted Buffer, Ponceau ready to use solution, diluted Destaining solution and Distilled Water in each tank. When present check the level of waste tank, it must be empty.

Strips Positioning

Place the strip on the strip holder as shown in the Operator's Manual. Do not touch the acetate side with fingers.

Samples

Dispense 25 µL of sample into each well of the sample plate. Avoid introducing air bubbles. Bubbles and foam can interfere with results.

Sample Plate

Place the blotter into the correct position as shown in the Operator's Manual. Load the sample plate onto the instrument.

Electrophoresis Conditions

Check on the instrument that the Serum Proteins migration conditions are correct for the specific test. Consult the Operator's Manual for further information on setting.

Start the run

Consult the operator's manual for further information about the starting procedure.

Additional note

Blotting Paper for migration chamber must be changed after 12 electrophoretic runs.
Blotting Paper for sample plate must be discarded after 5 runs.

Test Results

Fig. 1. Normal five fraction serum protein pattern on cellulose acetate. From the start point (cathodic edge) of the cellulose acetate strip to the anode, the bands are: Gamma Globulin, Beta Globulin, Alpha 2 Globulin, Alpha 1 Globulin and Albumin. It should be specified that, with conventional serum proteins dyes, a slight or mild staining of the background between the major fractions may occur due to the presence of lipoproteins, whose protein moieties can bind to the dye. In a normal pattern, Albumin, Alpha 1 and Beta fractions appear to be homogeneous, well shaped bands. Alpha 2 and Gamma fractions are diffuse bands with the Gamma fraction showing a more intensely stained zone in its central part.

Fig.1 SPE electrophoresis and position of bands

- start point (application zone)
- Gamma
- Beta
- Alpha 2
- Alpha 1
- Albumin

Reference Values

In the following table, the serum reference ranges were established with SPE kit on **SELEO type** instrument. These values are presented as a guideline. Each laboratory should establish its own reference values.

FRACTION	REFERENCE RANGE (%)
Albumin	52.0 - 68.0
Alpha 1	2.0 - 5.0
Alpha 2	6.5 - 13.5
Beta	8.5 - 14.5
Gamma	11.0 - 21.0

Quality controls

It is recommended to include controls with the patient sample runs in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. The laboratory should establish acceptance parameters for each lot of control material. If the controls are not within these parameters, patient test results are suspect and the analysis should be repeated.

Bibliography

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Running Conditions

Step	EXPRIME 72	ADALYA 24	GIANTS HS
Constant Current or Constant Voltage	10 mA or 165 Volt	10 mA or 165 Volt	10 mA or 165 Volt
Optical compensation	40 - 50	40 - 50	40 - 50
BUFFERING	180 SEC	180 SEC	180 SEC
DRYING 1✳	90 SEC	90 SEC	90 SEC
MIGRATION TIME	720 SEC	720 SEC	720 SEC
STAINING TIME	180 SEC	180 SEC	180 SEC
DESTAINING 1	360 SEC	360 SEC	360 SEC
DESTAINING 2	240 SEC	240 SEC	240 SEC
DRYING 2	15 SEC	15 SEC	15 SEC
APPLICATION OF SAMPLE TIME	20	20	20

✳ The drying time depends on moisture and temperature of the working area. During the application of samples step check the status of the surface of the plate. Increase or decrease the drying value in case the surface of the plate is too wet (drops of buffer) or too dry (white spots). The best results are achieved with a uniformly wet surface of the gel.

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The compliance of the kit with the directive 98/79/CE and related performance described in the procedure, are guaranteed only if the products are used according to our instructions of use

SAFETY CLAIMS

1.Before starting the assay, read the instructions completely and carefully. Be sure that everything is understood.
2.Don't mix reagents of different lots. Don't use expired reagents.
3.Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses.
4.If the reagents contained in the kit have an hazardous label, it may cause eye and skin irritations. MSDS for this product are available upon written request to the manufacturer.
5.Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.

SYMBOLS AND MEANING		
REF PRODUCT CODE	LOT LOT NUMBER	READ THE INSTRUCTIONS
EXPIRATION DATE	STORAGE TEMPERATURE	MANUFACTURER
DO NOT RE-USE	IVD IN VITRO DIAGNOSTIC	CE MARK
CONF CONFECTION		