Quality control

To control the quality of each electrophoretic run you should run one lane with a control serum. The corresponding values to reach are given in the products information leavelet.

Interpretation or results

It is recommended that any evaluation of the gels is performed against normal values produced for this method in each individual laboratory.
For a complete review of serum proteine evaluation, see Rithmann, S.E. 1982. Studies show that the values are the same for both males and non-pregnant females. Some differences are seen in pregnant females at term and women on oral contraceptives.
Age has some effect on normal levels. Cord blood has a decreased total proteon, albumin, alpha2 and beta fractions; slightly increased alpha 1 and normal or increased gamma fraction (largely of maternal origin). Tha gamma globulins drop rapidly until about 3 months of age, while other fractions have reached adult levels by this time. Adult levels of the gamma globulins are not reached until 10-16 years of age. The albumin decreases and beta globulin increases over the age of 40.

1. **Qualitative check:** Electrophoresis pattern has to be aproved for additional or missing bands.
2. **Quantitative check:** The calculated values by densitometer have to be aproved against normal values.

In all cases for values outside the normal range must be run further laboratory and analysis tests.

Limitation

Since all electrophoresis procedures are non-linear, it is important to follow these instructions for use closely to ensure optimal resolution and reproducible results. Failure to follow these instructions for use may affect the results obtained.

Reference values

Normal values (only valid for center of Europe!)

Fraktion	Normal range*
Albumin	57,0 – 68,0
Alpha 1	1,5 – 6,0
Alpha 2	5,0 – 11,0
Beta	7,0 – 13,0
Gamma	10,0 – 18,0

* Determined by Univ. Klinik Giessen, 1999

Literature / Support

1. Alper, C.A.'Plasma Protein Measurements as a Diagnostic Aid', N. Eng. J. Med; 1974; 291 : 287-290.
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3. Ritzmann, S.E. and Daniels, J.C. ‚Diagnostic Proteinology: Separation and Characterization of Proteins, Qualitative and Quantitative Assays' in Laboratory Medicine, Harper and Row. Inc., Hagerstown, 1979
4. Tietz, N.W. (Ed.), Textbook of clinical Chemistry, W.B. Saunders Co., Philadelphia, page 579 – 582, 1986
5. Ritzmann, S.E. (Ed.), Protein Abnormalities Volume I : ‚Physiology of Immunoglobulins – Diagnostic and Clinical Aspects', Allen R. Liss Inc. New York, 1982
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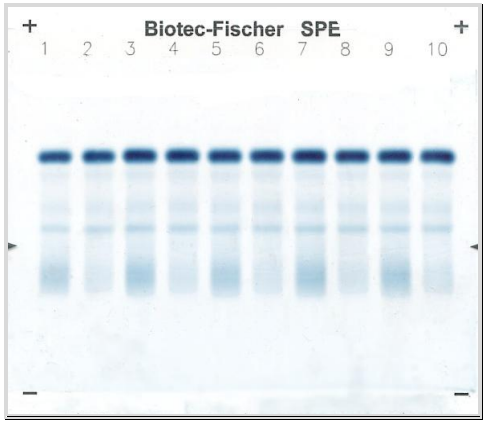

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Manual

SPE Electrophoresis on Agarosegel

for product code 370601 / 370602

Intend purpose

The folllowing named reagents are only used to migrate serum proteines on agarose gels

Human serum contains more than 100 single proteines which every single proteine has its specific function which depends on different physiological conditions.
Since the introduction of moving boundary electrophoresis by Tiselius, and the subsequent use of zone electrophoresis, serum proteines have been fractionated on tha basis of their charge at a particular pH. With BIOTEC-FISCHER serum proteine system the human proteines will be separated to their 5 main classes.. (Albumin, Alpha-1 globulin, Alpha-2 globulin, Beta globulin and Gamma globulin) according to the charge in BIOTEC-FISCHER agarose gels. After migration the proteines are stained to visualise them. Each of the classical electrophoretic zones, except Albumin, represents normally 2 or more proteine components. The relative portions of these fractions have proven to be useful aids in the diagnosis and prognosis of certain disease states.

Warning

All reagents are for in vitro diagnostic use only. Don not ingest or pipette by mouth any component. Refer to the product safety sheet for component risk and safety phrases in disposal information.

Buffer contains sodiumazide. Do not mix acid solution with sodiumazide to avoid toxic vapours. Always rinse abundantly with water to avoid the formation of metal-azides, because of the potential risk of explosion when piled up in metal conduct-pipes..

Necessary Equipment

Reagents

Prod. No.:	Name
370601	**Kit for 100 SPE Electrophoresis on Agarosegel** Content: 1 x 10 agarose gels for 10 tests per gel 1 x 10 thin sheet drying paper for agarose gels 1 x 10 sample templates 1 x 10 drying paper for sample templates 1 x 100 ml ATX buffer concentrate *Fill up one bottle to 1000 ml with Aqua dest.* 1 x 50 ml Amido Black staining concentrate *Dilute one bottle with 200 ml Aqua dest.*

Equipment

Prod. no.:	Name
321001	Electrophoresis tank model 1000
321000	*Electrophoresis tank model BFA (altern.)*
321101	*Electrophoresis tank FILIPO*
341225	Staining vessel kit for agarose gels
332010	Drying oven KB19
313001	MAESTRO 101 (no further equipment necessary)
331000	Densitometer TurboScan

Reagents which are not part of kit

Name
Fixativ
Ethanol : dist. Water : Acetic acid (50 : 40 : 10)
Destaining
3% Acetic acid

Shelf live

Unopened agarose gels will be usable until shelf live marking on the envelope

Sample preparation

Freshly collected serum is the specimen of choice. Samples can be stored at 15...30°'C up to 4 days, 2…6°C for up to 2 weeks or 6 months at –20°C. Urine and CSF can also be used following a suitable concentration step (50 – 100X). The use of plasma will result in a fibrinogen band between beta and gamma fractions.

Interfering factors:

1) Hemolysis may cause false elevation in the alpha 2 and beta fractions.
2) Inaccurate results may be obtained on specimens left uncovered, due to evaporation.

Sample preparation:
Dilute each sample 1 : 7 with buffersolution or 0.9% NaCl. (10µl serum + 60µl dilution solution)

Step by step procedure

1. Fill first vessel with fixative, second vessel with staining solution and the last two vessels with destaining solution.
2. Fill one compartment of tank model 1000 with buffer solution until it flows into the second compartment.
3. Tilt the tank so both compartments will be leveled (For Filipo and MAESTRO 101: fill both electrode compatments with 30ml buffer each. For tank model BFA 100 ml for each compartment).
4. Dry middle separator with paper towel
5. Carefully cut gel envelope on opposite small side of label with scissor and carefully remove the gel. Don't press to hard not to damage the gel. Position the gel in front of you so you can read the gel information
6. Carefully remove blue protection foil
7. Place one thin paperwick onto the gel for approx. 1-2 seconds and remove it afterward carefully
8. Position the sample template with small marking holes to the markers (▶ and ◀).
9. Place small paperwick in the middle of template and move your finger over the wick to remove air bubbles between template and gel.
10. Applicate 3 - 5µl sample to each application slid
11. Wait for 5 minutes until the sample intruded into the gel.
12. Place the small paper wick again onto the template to remove remaining sample and remove template together with paper wick and give it to the waste.
13. Place gel into the gel bridge with gelside down. Care about correct electrical positioning ("-" = black; "+" = red).
14. Move gel bridge into the tank. Care about correct electrical positioning!
15. Close tank lid
16. Run migration at 90 Volts for 30 minutes
17. Remove after migration and give it for 5 minutes in first vessel with fixation solution
18. Dry gel at 80°C in drying oven KB19 or use hair dryer.
19. After gel is completely dry give it for 5 minutes to staining solution
20. Move gel to remaining destaining vessel for 5 minutes each at last until there is no more visible background.
21. Again dry the gel like mentioned under point 18.
22. Calculate Gel with TurboScan densitometer