MANUAL OF PROTEIN ELECTROPHORESIS

The Hellabio Agarose Gels for protein electrophoresis are intended to be used for in vitro diagnosis, and they enable the quantitative and qualitative estimation of proteins in serum and other biological materials.

HELLABIO KITS [the size of the gels is 76X101mm)

Required Reagents and Equipment:

INCLUDED IN EACH KIT	
• Agarose Gels (10)	• Staining Solution (for 250 ml)
• Electrophoresis Buffer (for 1or 3 L)	• Sample Templates (10)
• Gel Blotter Strips (20)	• Diluent Solution
• Manual	

Additional reagents and equipment required and can be provided by Hellabio:

Power supply, Electrophoresis Tank, staining-distaining baths, Hellabio Scan (Gel Analyzer).

Reactive Ingredients:

Agarose gels: Agarose Gels in Tris-Hippuric buffer, 0.1% NaN3*, and other non-reactive ingredients for long stability and optimum resolution of protein fractions.
Diluent Solution: Tris-Hippuric acid pH 8.6+/- 0.2 + Bromphenol blue + 0.1% NaN3*
Buffer: Tris-Hippuric acid buffer, pH 8.6+/- 0.2 + 0.1% NaN3*
***NaN3 may form explosive compounds in metal drain lines.**

Staining Solution:

Concentrated Amido Black solution in 10% acetic acid. To use dilute it according to instruction on the bottle to get the working solution.

Controls:

Pooled sera or commercially available quality control sera can be included in each electrophoresis procedure.

Interpretation of the results:

The qualitative interpretation of the results may be visually interpreted by comparing the sample pattern with the control pattern. For a quantitative interpretation of the result HellabioScan or densitometer (520-600nm) can be used.

Limitation / Caution:

Do not use the agarose gels if it seems to be dried.
Do not freeze the agarose gels.
Store the kit in horizontal position.
Avoid using hemolytic sera or plasma (fibrinogen)

Calibration: The estimation of the electrophorogram for paraproteinemia can be easily done visually. For a quantitative analysis HellabioScan or densitometer (520 - 600nm) can be used.

Special considerations : Drugs that can affect the measurement of total proteins in serum and in urine include chlorpromazine, corticosteroids, isoniazid, neomycin, phenacemide, salicylates, sulfonamides, and tolbutamide.

Preparation of reagents, storage and stability:

Agarose gels: The Gels must be storage at room temperature in horizontal position until the expiration date. Do not freeze the gels. Just before use carefully discover the gel and follow the instruction of the manual.

Diluent Solution: Store it at room temperature. For 1:4 dilution of the serum add to 1 volume of serum 3 volumes of diluent solution.

Electrophoresis Buffer:

The concentrated buffer should be stored at room temperature until the expiration date. If there appear any crystals as sediment, put it in warm water bath to dissolve the crystals. To use dilute the content of the bottle in deionised water according to the instruction on the bottle. The reconstituted buffer can be stored at room temperature in a closed flask until 3 months.

Controls: Store it at 2-8^{0}C. Dilute it according the instruction.

Staining Solution:

Store the concentrated solution at room temperature until the expiration date. To use dilute the content of the bottle with 10% acetic acid according to the instruction on the bottle to get the working solution. The working solution is enough for the staining of 10 gels. It should be stored in a good closed flask at room temperature until 3 months.

Distaining Solution:

Three subsequently baths of 10% acetic acid solution. Store it at room temperature.

Steps of Protein Electrophoresis

1. Dilute freshly serum sample 1:4 with protein diluent solutions (10µl+30µl).
2. Fill the electrophoresis chamber with adequate volume (it depends on the chambers volume) of electrophoresis buffer.
3. Take the agarose gel out of its packaging, uncover it from the plastic plate and put it on a horizontal position.
4. Blot the gel on the sample application zone with a gel blotter strip.
5. Place the suitable sample template on the application zone.
6. Rub the template with forefinger so that it gets contact with the gel surface and no air bubbles exist.
7. Using a 5 µl pipette apply 5 µl of serum dilution across each corresponding slit and let them absorb into the gel 2 mins. The application of the samples should be done as quick as possible. The samplest should not be allowed to dry.
8. Blot the excess sample with a gel blotter strip, gently remove both the sample template and gel blotter strip and discard them.
9. Place the gel into the tank with the samples on the cathodic side, connect the tank to the power supply and run 20 mins /100 Volts (the running time depends on the power supply)..
10. Dry the gel completely (the proteins will be fixed in agarose) with hot air (less than 60°C) and stain it for 5 mins with protein staining solution.
11. Distain the gel for 5 mins in three-distaining solution baths, subsequently.
12. Dry again the gel with hot air and evaluate the results by HellabioScan or densitometer using 520-600 nm filter.

Expected values:

The values presented are those for serum protein electrophoresis on the Hellabio agarose gel. The normal range values for Hellabio Agarose gels were determined by calculating the mean value +/-2 standard deviation for each protein fraction from a population of 200 apparently healthy male and female adult blood donors from North Greece.

Anyway it is recommended for each laboratory to establish its own normal range values in its own densitometer just one time.

Proteins fraction	Normal % of total proteins
Albumin	52.0 – 65.0
α_1 -	2.0 - 5.5
α_2 -	6.0 - 11,7
β -	8.2 - 14,7
γ-globulin's	9.5 – 19.8

Precision: In a within-gel electrophoresis of 10 replicated position of a control serum the coefficient of variation values is about 5%.

Normal –Abnormal Values of Proteins

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