PROTEIN ELECTROPHORESIS
on agarose Gels

IVD CE

Principles and Methodology

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 fluids.
The clinical use of Electrophoresis in protein analysis generally is based on the simple electrophoretic separation of proteins according to their relative mobility and molecular weight , into albumin, α_1-, α_2-, β-(β_1, β_2), and γ- globulins in spite of the knowledge that each of the classical electrophoretic zones may contain more than one major proteins.

Required Reagents and Equipment's included in each kit:

[Warning: All reagents from each kit must be used together]

Product	KIT PE30 / 300 TESTS
Agarose Gels	**10**
Electrophoresis Buffer [50X concentrated]	**20 ml**
Staining Solution [5X concentrated]	**60 ml**
Protein Diluent Solution [ready to use]	**31 ml**
Destaining solution [500X concentrated]	**8 ml**
Gel Blotter Strips	**40**
Sample Templates	**20**
Instructions for use in English	

All reagents must be used according to the instructions and until the expiration date indicated on the kit

Preparation, storage and stability of the reagents included in the kit:

a) Agarose Gels: Agarose Gels are in non- barbital buffer and other non-reactive ingredients for long stability and optimum resolution of protein fractions. The Gels must be stored at **15 - 25 ^{0}C on horizontal position** until the expiration date indicated on the kit. Do not freeze the gels. Carefully discover the gel just before use and follow the instructions of the manual.
b) Diluent Solution: Working electrophoresis buffer + Bromphenol blue and other non-reactive ingredients. Store it at 15 - 25 ^{0}C until the expiration date indicated on the kit. **Ready to use**.
c) Electrophoresis Buffer: Non-barbital buffer and other important non-reactive ingredients. It is in concentrated solution. It must be stored at 15 – 25 ^{0}C until the expiration date indicated on the kit. **To prepare working solution dilute the content of the bottle with deionized water to a final volume of 1 litre.** The diluted solution is enough for electrophoresis of all gels of the kit. **The buffer solution is for one use only**. Store the diluted solution at room temperature until the expiration date indicated on the kit.
If crystals appear, place the vial in warm water to dissolve the crystals.
d) Staining Solution: Acetic acid free concentrated Amido Black solution. Store the concentrated solution at 15-25 ^{0}C until the expiration date indicated on the kit. **To prepare working solution dilute the content of the bottle with deionized water to a final volume of 300 ml(PE kits) or 150 ml(MPE kits).** The diluted solution is enough for the staining of all gels of the kit. It should be stored in a closed flask at room temperature until the expiration date indicated on the kit.
e) Gel Blotter strips: Thin filter paper strips to blot the gel in the application area. Blot just for 5 seconds. Avoid humidity.
f) Destaining solution: 2% citric acid solution. **To prepare working solution dilute 2 ml of the content of the bottle with deionized water to a final volume of 1 litre**. Store at room temperature until the expiration date indicated on the kit.

Additional Reagents and Equipments which can be provided by Hellabio:

Controls: Pooled sera or commercially available quality control sera can be included in each electrophoresis procedure.
Power supply, Electrophoresis tank, Staining-destaining baths, HellabioScan (Gel Analyzer).

Limitation / Caution:

- *Do not use the Agarose gel if it seems to be dried or infected by fungi.*
- *Do not freeze the agarose gel.*
- *Store the kit on horizontal position.*
- *Do not use hemolytic sera or plasma.*

Collection and handling of specimens:

The samples should be collected according to the standard hospital methods.

a) Blood: *Analysis is preferably performed on **sera** in order to avoid the fibrinogen band (in plasma) which migrates in the gamma zone and could lead to a false interpretation. Venous blood from a fasting individual is drawn into a tube. After centrifugation the supernatant fluid (serum) can be stored at 4-8°C for 48-72 hours. Storage at -20°C may disturb some of the electrophoretic patterns. It is known that the proteins are stable for 2-3 days at 4°C. However, other serum components such as lipoproteins and isoenzymes are unstable and therefore, they have to be tested within 1-2 days. Care should be taken to prevent haemolysis in the serum because it will cause false elevation in the α_2- and β-fractions.*

b) Cerebrospinal fluid (CSF): *Normally, some 80% of the proteins of cerebrospinal fluid originate from plasma, and the rest have been synthesized locally. The filtration resistance for plasma protein rapidly increases with molecular size and therefore the ratio of large proteins normally is much lower in CSF than in plasma. The protein content of CSF is in the range of 15-40 mg/dl. Therefore it must be concentrated at least 150-fold before electrophoresis.*

c) Urine: *Analysis of the urinary proteins shows that normal urine contains more kind of proteins, which are much diluted, and therefore it must be concentrated. For the detection of Bence-Jones proteins, free Kappa and Lambda light chains the sample has to be concentrated to ≥ 100 mg/dl of total protein and to about 80 g/dl of total proteins for immunoglobulins.*

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 a densitometer (520nm-600nm) can be used.

Procedure of Protein Electrophoresis

a) Dilute fresh serum (not plasma) sample 1:6 with protein diluent solution (20 µl serum+100µl diluent solution).

b) Fill the electrophoresis chamber with adequate volume (it depends on the chamber's volume) of electrophoresis buffer.

c) Take the Agarose gel out of its packaging, uncover it from the plastic plate and put it on the backside of the plate in horizontal position.

d) Blot the gel for 5'' with a gel blotter strip on the sample application zone.

e) Place the suitable sample template on the application zone.

f) Rub the template with the forefinger so that it gets contact with the gel surface.

g) Using a 5 µl pipette, apply 5 µl of serum dilution across each corresponding slit and let them absorb into the gel for 2 minutes.

h) Blot the excess sample with a gel blotter strip, gently remove both the sample template and the gel blotter strip and discard them.

i) Place the gel on the gel carrier with the gel **upstairs** and the samples on the cathodic side (-), put it in the tank, connect the tank to the power supply and run 18 minutes /100 Volts.

j) Dry the gel completely (the proteins will be fixed in Agarose) with hot air (less than 90°C) and stain it for 5 minutes with protein staining solution.

k) Decolonize the gel for 5 minutes in three-destaining solution baths subsequently.

l) Dry again the gel with hot air, **clean well the backside of the film** and evaluate the results by HellabioScan or by a densitometer using 520-600 nm filters.

Expected values:

The values presented in the table 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 [Serum of a population of 200 apparently healthy male and female adult blood donors from Macedonia, in North Greece, are used].

Anyway it is recommended for each laboratory to establish its own normal range values in its own analysis system (HellabioScan or a densitometer) just one time.

Proteins fraction	Normal %
Albumin	52.0 - 65.0
α_1 -globulins	2.0 - 5.5
α_2 - globulins	6.0 - 11,7
β_1 - globulins	4.9 - 9.9
β_2 - globulins	3.3 - 5.3
γ-globulins	9.5 - 19.8

Reproducibility within run

Three different samples were each run in 10 tracks on Hellabio PE10 gels from the same lot. The means, SD and CV (n = 10) were calculated for each serum sample and each zone. The following table shows results for the 3 serum samples.

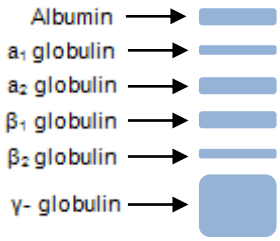
Fraction	MV (%)			SD			CV (%)		
Sample	1	2	3	1	2	3	1	2	3
Albumin	55,90	59,83	61,77	2,61	3,26	3,89	0,046	0,054	0,062
α1-	4,78	3,68	2,06	0,71	1,08	0,71	0,148	0,294	0,345
α2-	7,90	6,09	7,12	1,12	1,98	1,80	0,141	0,325	0,252
β-	14,05	11,27	16,52	2,09	1,27	1,78	0,148	0,112	0,107
γ-globulin	17,37	19,12	12,52	2,53	2,40	1,32	0,145	0,125	0,105

ACCURACY

45 different sera were run on Hellabio system [agarose gel PE10 and analysis by HellabioScan] and on other commercially system [agarose gel and analysis software].
The correlation parameters calculated for individual zones on Hellabio system vs. the comparative gel system was:

Fractions	Correlation Coefficient	γ-Intercept	Slope	Range of % value of samples used in Hellabio Gel
Albumin	0.933	2.867	0.963	49.0 – 74.6
α1-	0.970	0.972	0.963	1.4 – 6.3
α2-	0.962	0.735	0.944	6.0 – 15.7
β-	0.973	0.003	0.967	7.2 – 18.2
γ-globulin	0.958	-0.545	0.988	7.5 – 19.8

Serum protein separation pattern



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