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- globulin's 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 PEA10 / 100 TESTS/KIT	KIT PEA13 / 130 TESTS/KIT	KIT PEA26 / 260 TESTS/KIT
Agarose Gels	10	10	10
Sponges	20	20	20
Buffer Solution [3X conc.]	50 ml	50 ml	50 ml
Staining Solution [5X conc.]	60 ml	60 ml	60 ml
Destaining solution [500X conc.]	20 ml	20 ml	20 ml
Gel Blotter Sheets	10	10	10
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) Electrophoresis Buffer for sponges: 3X concentrated stock solution of non barbital buffer and important non-reactive ingredients. To prepare working solution dilute it 1:3 [dilute the buffer to 100ml distilled water]. To use put the sponges on the plastic plate on horizontal position and add to each sponge 6.5 ml of working buffer solution. The working buffer solution can be stored at 4-8 ^{0}C until the expiration date indicated on the kit.
c) 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 according to the instructions on the bottle. 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 3 months.
e) Gel Blotter sheets: Thin filter paper strips to blot the gel. Blot just for 5 seconds. Avoid humidity.
f) Destaining solution: 2% citric acid solution. To prepare working solution dilute the content of the bottle according to the instructions on the bottle. 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.

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.*

Procedure of automatic Protein Electrophoresis

01. Prepare all the reagents (staining-destaining solution) according to the instructions and fill the staining and destaining tank.
02. Fill the distilled water tank with distilled water and 100 ml of tap water.
03. Check that the waste tank is empty.
04. Turn on your computer.
05. Turn on the general switch located on the left backside of the instrument.
06. Then press the power button on the front of the machine (Blue button).
07. Lift the cover of the machine.
08. Take out the sponges from their packaging. Add 6.5 ml buffer on each sponge and apply them on the migration chamber slots.
09. Locate the migration chamber in correct position.
10. Transfer 20-30μl of each sample to the holes of the sample holder.
11. Take the gel out of the plate and put it on the back side of the plate.
12. Blot gently for 5 seconds the surface of the gel with one gel blotter sheet.

13. Apply 0,5ml distilled water on the electrophoresis area.
14. Remove the gel holder and the gel hold support.
15. Fasten the film on the gel holder on the side marked **+ (anode)**. Apply the film on the electrophoresis area.
16. Apply carefully the gel hold support on the gel.
17. Double Click on Gemini software.
18. Click on LOGIN**.**
19. Click on METHODS.
20. Select the type of electrophoresis (Serum).
21. Adjust the parameters:

	Kit					
	PEA10, PEA13			PEA26		
Phase	Time	^{0}C	mA	Time	^{0}C	mA
Premigration	3'	20	-	3'	20	-
Sampling samples	20''	-	-	20''	-	-
Deposition samples	20''	-	-	20''	-	-
Migration	9'	20	190	9'	20	190
Desiccation	15'	75	-	15'	75	-
Staining	4'	-	-	4'	-	-
Destaining (9 cycles)	6'09''	-	-	6'09''	-	-

22. Save the parameters.
23. Click on the option New Analysis.
24. Select serum protein electrophoresis.
25. Choose the kind of the strip (gel).
26. Choose the number of the samples to be analyzed.
27. Click on the option Confirm.
28. Enter the patient's data. Click on the option SAVE for each patient.
29. Click on the option START ANALYSIS.
30. Check the status of the machine.
31. Click on the option Continue the process.

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.

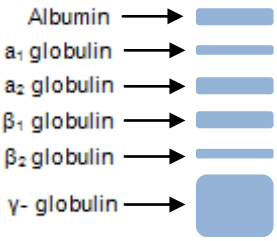
Fraction	MV (%)			SD			CV (%)		
Sample	1	2	3	1	2	3	1	2	3
Albumin	55,90	59,83	61,77	2,61	3,26	3,89	0,046	0,054	0,062
α1-	4,78	3,68	2,06	0,71	1,08	0,71	0,148	0,294	0,345
α2-	7,90	6,09	7,12	1,12	1,98	1,80	0,141	0,325	0,252
β-	14,05	11,27	16,52	2,09	1,27	1,78	0,148	0,112	0,107
γ-globulin	17,37	19,12	12,52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

Exemplary serum protein separation



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