



## ELECTROPHORESIS OF LIPOPROTEINS

Lipoproteins are the molecules which carry cholesterol and triglycerides throughout the body. The excess particular of cholesterol, can put a person at serious risk for cardiovascular disease. Electrophoresis is an easy and practical way to analyse the type and the concentration of lipoproteins.

There are four major classes of lipoproteins:

- Chylomicrons.
- Very low-density lipoproteins
- Low-density lipoproteins, also called “bad cholesterol.”
- High-density lipoproteins, also called “good cholesterol

*The determination of serum lipoproteins has been utilized in studies on the genesis of atherosclerosis, response to hormonal levels, congenital and developing anomalies and genotypes. Lipoproteins differ in molecular size and because of their negative charge, at pH 8.6, they migrate, in an electrical field, toward the anode.*

*Agarose gel electrophoresis, in comparison to other techniques, requires less time for completion and is very useful because the migration rate of the lipoproteins is much more reproducible from run to run.*

*HELLABIO Agarose Gel Films enable the separation of lipoproteins in chylomicron,  $\beta$ -, pre- $\beta$ -, (a) and  $\alpha$ -lipoproteins.*

### LIPOPROTEIN ELECTROPHORESIS KITS

#### INCLUDED IN KIT

Agarose Gels (10 )  
Electrophoresis Buffer( for 1or 3 L)  
Gel Blotter Strips (20)  
Sample Templates (10)  
Lp-Staining Solution (Stock solution)  
NaOH 0.1M

#### Preparation of Reagents:

- Electrophoresis Buffer:  
Dilute the content of the bottle in deionised water according to the instruction on the bottle. Store it in a closed flask at room temperature
- Working LE Staining Solution:  
Add 5 ml of the stock solution in a glass flask and add drop wise to it under continues mixing 1 ml 0.1 M NaOH.
- Clearing Solution: Mix thoroughly 7 vol. methanol with 3 vol. of deionised water. Store it at room temperature in a closed flask.

## Collection and Handling of Specimens:

Collect 12 hours fasting blood specimens in a vacutainer tube containing 1.5 mg Na<sub>2</sub> EDTA per ml blood. Do not use heparin because of its adverse effect on the lipoproteins pattern. Serum can be used too.

The Lipoproteins are generally instable. To protect them the serum or plasma should be separated from the red blood cells within 2 hours after collection.

The  $\beta$ - and  $\alpha$ -lipoproteins remain relatively constant over at lest 28 days at 4 °C. However, there is a decrease in the mobility of the pre- $\beta$ -lipoproteins. The change is most rapid in the first five days.






Freezing of the plasma irreversibly alters the lipoprotein pattern and therefore it must be stored at 4 °C.

Ideally electrophoresis of LP should he performed on the day of collection.

## Procedure of Lp –Electrophoresis:

1. Dilute freshly serum sample 1:2 with Proteins diluent solution.
2. Fill the electrophoresis chamber with adequate volume (it depends on the chamber volume) of electrophoresis buffer.
3. Take the agarose gel film out of its packaging, uncover it from the plastic plate and put it on a horizontal position.
4. Blot the gel film with a gel blotter strip on the sample application zone.
5. Place the sample template on the application zone. Rub the template with forefinger so that it gets contact with the gel surface and no air bubbles exist.
6. Using a 5  $\mu$ l pipette, apply 5  $\mu$ l of serum dilution's across each corresponding slit and let them absorb into the gel for 5 mins. The application of the samples should be done as quick as possible. The application slits should not be allowed to dry.
7. Blot the excess sample with a gel blotter strip and gently remove both the sample template and gel blotter strip and discard them.
8. Place the gel film into the tank in the right charge position, connect the tank to the power supply and run 25 mins 100 Volt.
9. Following the electrophoresis, switch off the power supply and dry completely the gel with hot air (less than 60°C).
10. Place the film on a horizontal position and dispense onto it 3 ml of working Lp-staining solution.
11. After 3 mins discard the stain and put the films in two baths of distaining solution for 2 mins.
12. Dry again the film and interpret the results visually or by densitometer using 520 nm filter.

## Expected values:

Lipoprotein	Expected migration position from anode to cathode	Normal value %
$\alpha$ -Lp		22 - 46
Lp(a)		--
Pre $\beta$ -Lp		0 - 27
$\beta$ -Lp		47 - 71
Chylomicron		--