CORMAY GEL PROTEIN 100

DIAGNOSTIC KIT FOR THE ELECTROPHORESIS OF SERUM PROTEINS ON AGAROSE

Kit name	Kit size	Cat. No
CORMAY GEL PROTEIN 100	100 separations	6-048

INTRODUCTION

CORMAY GEL PROTEIN 100 is designed for electrophoretic separation of serum proteins on agarose. It permits to obtain six serum proteins fractions:
- albumin,
- alpha-1 globulins,
- alpha-2 globulins,
- beta-1 globulins,
- beta-2 globulins
- gamma globulins

METHOD PRINCIPLE

Protein electrophoresis is a well established technique routinely used in clinical laboratories for screening of serum. It is based on the principles of zone electrophoresis performed on a suitable support medium. The separated proteins are fixed in acid/ alcohol mix and stained with amidoblack solution. The excess of stain is removed with an acid solution. The stained electrophoretic separations can be evaluated visually for pattern abnormalities or by densitometry to obtain accurate relative quantification of individual zones.

PACKAGE

	CORMAY GEL PROTEIN 100
Agarose gel (plates with agarose)	10 pcs.
TRIS-BARBITAL buffer (concentrated)	3 x 100 ml
AMIDOBLACK staining solution (concentrated)	1 x 100 ml
Destaining solution (concentrated)	1 x 100 ml
Blotters	20 pcs.
Templates for sample application	10 pcs.

Store at room temperature in horizontal position (gel upside down), tightly closed. Do not freeze!

Reagent preparation

Buffer: 1 vial of concentrated buffer (TRIS-BARBITAL) to be diluted to 1000 ml with distilled water.
After dilution buffer stored at room temperature is stable until expiry date printed on the vial.
Staining solution: 1 vial of concentrated solution (AMIDOBLACK) to be diluted to 300 ml with distilled water.
After dilution staining solution stored at room temperature is stable until expiry date printed on the vial.
10 gels can be stained with 300 ml of diluted staining solution.
Destaining solution: 1 vial of concentrated destaining solution to be diluted to 10 litres with distilled water (or 10 ml to 1000 ml).
Stability: 1 month at room temperature.
Fixative solution: mix 135 ml of ethanol 96 %
30 ml of glacial acetic acid
135 ml of distilled water

or

135 ml of ethanol 96 %
37.5 ml of acetic acid 80%
127.5 ml of distilled water

Fixative solution must be prepared at least 15 min. before use.
Stability: 3 months at room temperature in tightly closed vial.
5 gels can be processed with 300 ml of fixative solution.
Note: Different concentrations of acetic acid and/or ethanol can be also used (e. g. acetic acid 80 %, ethanol 70 %). However, the proportion of reagents and distilled water used for the preparation of fixative solution should be changed in order to keep the quantitative composition of fixative solution constant.
Agarose gels: ready to use.

Warnings and notes

- Product for in vitro diagnostic use only.
- During electrophoresis, buffer may turn yellow to brown with slightly brown sediment without any adverse effects on its performance
- Reagent Tris-barbital buffer is classified as a toxic!

Ingredients: barbital, sodium barbital, sodium azide
T – Toxic.
R 22-42/43-61 – Harmful if swallowed. May cause sensitization by inhalation and skin contact. May cause harm to the unborn child.
S 1-36/37/39 – Keep locked up. Wear suitable protective clothing, gloves and eye/face protection.

- Reagent Amidoblack solution is classified as a corrosive!

Ingredients: acetic acid
C – Corrosive.
R 34 – Causes burns.
S 23-26 - Do not breathe vapour / spray. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

ADDITIONAL EQUIPMENT

- power supply and Cormay equipment for electrophoresis or Beckman electrophoresis system;
- glacial acetic acid, ethanol 96% for fixative solution preparation;
- general laboratory equipment;

SPECIMEN

Serum diluted with diluted buffer (1 volume of serum + 6 volumes of buffer). Native serum can be stored at 2-8°C up to 3 days. Serum should be diluted just before use.

PROCEDURE

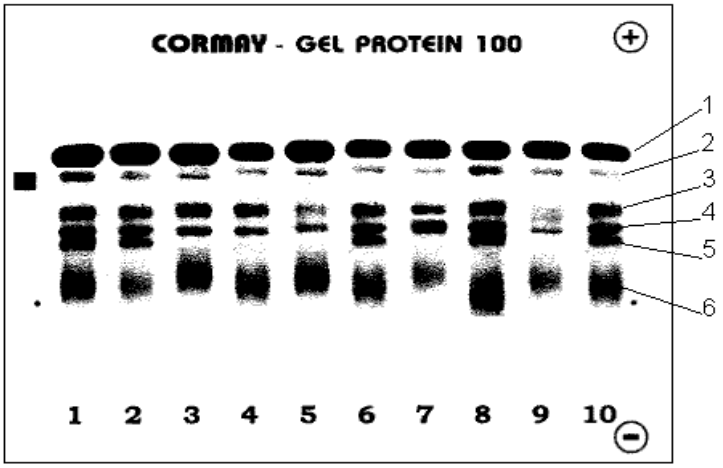
1. Pour 150 ml (Cormay CU-1, Cormay S20) or 45 ml (Beckman) of diluted buffer into each part of chamber.
2. Take the gel out of package not touching its surface and place it on a sheet of paper or blotter.
   To avoid gel drying and connected with this analytical problems open the foil bag immediately before use, when the whole equipment is ready for use and sera are diluted.
3. Use blotter to dry area where samples will be placed. Remove wet blotter immediately.
4. Place the sample template on the gel (two edge slots of template to be connected with two orientation signs on the plate). Move finger longwise the template to press it to the gel. Template should firmly adhere to the gel.
5. Apply 5 µl of diluted serum to each slit and leave it for 5 min. since the application of the last sample.
6. Remove excess of serum with blotter.
7. Take the sample template off.
8. Curve agarose plate and place it on the bridge **upside down,** samples on the cathodic side (-).
9. Close chamber with cover.
10. Perform electrophoresis for 20 min. at 100 V (Cormay CU-1, Cormay S20) or for 15 min. at 100 V (Beckman).
11. After migration remove plates and immerse them in the fixative solution for 15 min. **in vertical position**.
12. Dry the gel under hot air stream or in the oven at the temp. up to 80°C until it is completely dry.

13. Immerse the plate in the staining solution for 10 minutes, then destain it in 2 - 3 successive baths of destaining solution.
14. Rinse the gel with distilled water and dry it with hot air up to 80°C.
15. Interpretation can be done visually or using densitometer.
16. If necessary, clean the back side of the plate with a wet paper.

INTERPRETATION OF RESULTS

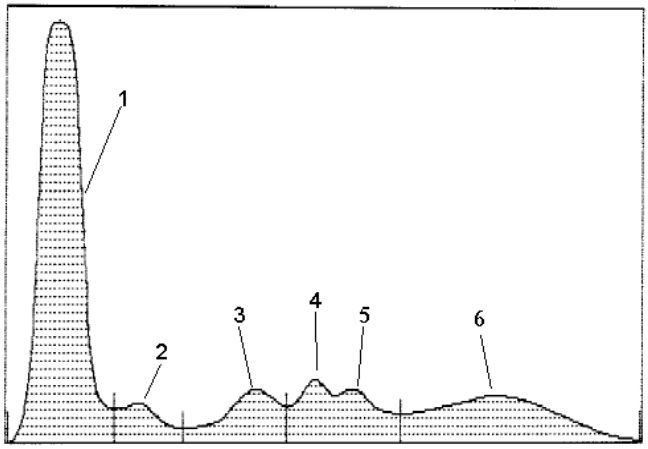
CORMAY GEL PROTEIN 100 gives perfect separation of serum proteins into 6 fractions. The presence of monoclonal proteins (mainly in gamma or beta) is the indication for the immunofixation.

Exemplary serum protein separation:



1 - albumin
2 - alpha-1 globulins
3 - alpha-2 globulins
4 - β-1
5 - β-2 } β-globulins
6 - γ-globulins

Exemplary densitogram of serum proteins:



Fraction:	Rel [%]
ALBUMIN	55.75
ALFA 1 - GLOBULINS	3.41
ALFA 2 - GLOBULINS	8.19
BETA 1 - GLOBULINS	8.17
BETA 2 - GLOBULINS	4.61
GAMMA - GLOBULINS	19.88

MEAN VALUES

albumin	50 – 62%
alpha-1 globulins	2.5 – 6.0%
alpha-2 globulins	6 - 12 %
beta-1 globulins	5 - 10 %
beta-2 globulins	3 – 7.5 %
gamma globulins	12 – 22 %

It is recommended for each laboratory to establish its own reference ranges for local population.

PERFORMANCE CHARACTERISTICS

- **Reproducibility**

Protein fraction	Reproducibility in gel	Reproducibility between gel
	CV%	CV%
albumin	1.0	3.0
alpha-1 globulins	4.3	5.4
alpha-2 globulins	2.2	3.4
beta-1 globulins	2.9	5.9
beta-2 globulins	3.8	7.0
gamma globulins	2.3	3.7

- **Sensitivity / Limit of Quantitation:** Sensitivity is assayed as the lowest concentration of proteins, which is visible as a streak – 0.03 g/l on streak.

WASTE MANAGEMENT

Please refer to local legal requirements.

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MANUFACTURER

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