

Quality control

To check the quality of migration it is recommended to run control sample on each gel. So you ensure more reliable interpretation of shown results.

Interpretation or results

It is recommended to compare the results against normal shapes and values. It may be necessary to evaluate laboratory internal normal values.

- 1. Qualitative check:** Electrophoresis pattern has to be approved for additional or missing bands.
- 2. Quantitative check:** The calculated values by densitometer have to be approved against normal values.

In all cases for values outside the normal range must be run further laboratory and analysis tests.

Restriction

Due to that electrophoresis is a non linear physical methode, you strictly should follow this manual to get valuable results.

Reference values

Normalwerte (only valid for center of Europe!)

Fraktion	Normal range
Heparansulfat	N/A
Dermatansulfat	N/A
Chondroitinsulfat	N/A
Keratansulfat	N/A

Literature / Support

1. Laboratory Guide to the Methods in Biochemical Genetics, Blau, N.; Duran, M.; Gibson, K.M.; Springer Verlag Germany, ISBN 978-3-540-76697-1
2. Herr Dr. Adrian C. Sewell, Klinikum der Joh. Wolfgang. Goethe Univ. Frankfurt
3. Frau Dr. Jeanette Klein, Labor Berlin, FB Endokrinologie & Stoffwechsel
4. Analytical Biochemistry, 119, p.120-127, 1982



For further questions please contact:

Biotec-Fischer GmbH
Daimlerstrasse 6
35447 Reiskirchen
Germany
Fon: +49-6408-6072
Fax: +49-6408-64165
Mail: info@biotec-fischer.de



Manual

MPS Electrophoresis on Agarosegel

for product code
379601-001

Intend purpose

Following reagents are designed to be used for electrophoresis or Glykosaminoglykanes on Agarose Gel only.

Glycosaminoglycanes (GAG) or Mucopolysaccharides (MPS) are acid polysachharides built from linear repetitive disaccharides. Singel disaccharide units contain Uronic acid (mostly Glucuronic acid, more seldom Iduronic acid, Uronic acid of Idose), which are connected by a 1-3-glycosidic connection with an aminosugar like N-Acetylglucosamin. The disaccharid-units of chain itself are connected 1-4-glykosidic. Partially the Glycosaminoglycanes are esterified with sulfuric acid or acetic acid.

Warning

All reagents are for In Vitro use only. Never aspirate reagents with your mouth. Consult MSDS of reagents befor use.

Necessary Equipment

Reagents

Prod. No.:	Name
379601-001	Kit for 100 Electrophoresis on Agarosegel Content: 1 x 10 agarose gels for 10 tests per gel 1 x 10 thin sheet drying paper for agarose gels 1 x 10 sample templates 1 x 10 drying paper for sample templates 1 x 1 Manual

Equipment

Prod. no.:	Name
321001	Electrophoresis tank model 1000
321000	<i>Electrophoresis tank model BFA (altern.)</i>
341225	Staining vessel kit for agarose gels
300000	Drying oven KB19
331000	Densitometer TurboScan

Reagents which are not part of kit

Name
Migration buffer <i>0.1 M Barium Acetate</i> <i>Adjust with Acetic Acid to pH 5.2 – 5.6</i>
Staining <i>1 gr. Toluidinbue in 100 ml 5%ige Acetic acid</i>
Fixativ <i>Ethanol : dist. Water : Acetic acid (50 : 40 : 10)</i>
Destaining <i>3% Acetic acid</i>

Shelf live

Unopened agarose gels will be usable until shelf live marking on the envelope

Sample preparation

Hoopwood 1982 [4]

Step by step procedure

1. Fill first vessel with fixative, second vessel with staining solution and the last two vessels with destaining solution.
2. Fill one compartment of tank model 1000 with buffer solution until it flows into the second compartment.
3. Tilt the tank so both compartments will be leveled (For Filipo and MAESTRO 101: fill both electrode compamtments with 30ml buffer each. For tank model BFA 100 ml for each compartment).
4. Dry middle separator with paper towel
5. Carefully cut gel envelope on opposite small side of label with scissor and carefully remove the gel. Don't press to hard not to damage the gel. Position the gel in front of you so you can read the gel information
6. Carefully remove blue protection foil
7. Place one thin paperwick onto the gel for approx. 1-2 seconds and remove it afterward carefully
8. Position the sample template with small marking holes to the markers (▶ and ◀).
9. Place small paperwick in the middle of template and move your finger over the wick to remove air bubbles between template and gel.
10. Applicate 3 - 5µl sample to each application slid
11. Wait for 5 minutes until the sample intruded into the gel.
12. Place the small paper wick again onto the template to remove remaining sample and remove template together with paper wick and give it to the waste.
13. Place gel into the gel bridge with gelside down. Care about correct electrical positioning ("-" = black; "+" = red).
14. Move gel bridge into the tank. Care about correct electrical positioning!
15. Close tank lid
16. Run migration at 50 Volts for 60 minutes
17. Remove after migration and give it for 5 minutes in first vessel with fixation solution
18. Dry gel at 80°C in drying oven KB19 or use hair dryer.
19. After gel is completely dry give it for 5 minutes to staining solution
20. Move gel to remaining destaining vessel for 5 minutes each at last until there is no more visible background.
21. Again dry the gel like mentioned under point 18.
22. Calculate Gel with TurboScan densitometer