Step by Step procedure (continued)

24. Place a thin blotting paper, soaked with saline solution, on top of the gel and add some thick filter papers on top (4-6 pcs). Place a glass plate and then a weight of approx. 1kg on top of it. Press this sandwich for approx 10 minutes . (The thin blotting paper and the following 3 thick blotting papers are given to the waste. The remaining thick blotting papers can be reused for repeating procedure).
25. Before removing the thin blotting paper from gel surface, give gel into saline solution for one second. Then remove the first thin blotting paper.
26. Place the gel for 2-3 minutes in fresh 0,85 % saline solution while gentle shaking.
27. Press again like described in point 24+25 but for 2 minutes only.
28. Dry the gel for approx. 10 minutes at ~50°C . 70°C hot air flow.
29. Place the gel for 4 minutes in staining solution
30. After staining place the gel into bath 3 and 4 (destaining solution each) for 3 minutes each. (Optionally you can dry and/or blot the gel again between the two destaining steps. This will decrease remaining background)
31. After destaining dry the gel again for approx. 10 minutes at ~50°C – 70°C hot dry airflow

Interpretation of results

It is recommended that any evaluation of the gels is performed against normal values produced for this method in each individual laboratory.

1. **Qualitative evaluation:** The electrophoresis may be inspected for the presence or ablence of particular bands of interest.
2. **Quantitative evaluation:** Not applicable for this method

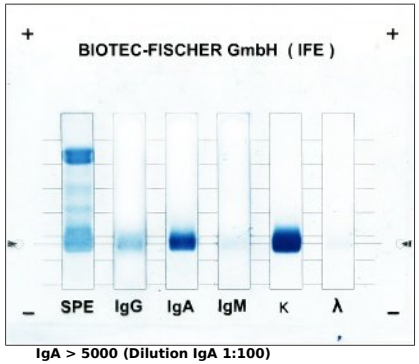
In either case, an elevation or decrease in particular serum components or the detection of unusual serum components require further investigation.

Limitation

Since all electrophoresis procedures are non-linear, it is important to follow these instructions for use closely to ensure optimal resolution and reproducible results. Failure to follow these instructions for use may affect the results obtained

Literature

1. Arcquembourg, P.C. Salvaggio J.E., Bickers J.N. (1970), Primer of immunoelectrophoresis with interpretation of pathologic human serum patterns, S. Karger, 6-29
2. Johnson A.M., Alper C.A. (1969, Immunofixation electrophoresis a technique for the study of protein polymorphism, Vox Sang 17, 445-452
3. Thompson R.A. (1981), Technique in clinical Immunobiology, 2nd edition, 1-27



IgA > 5000 (Dilution IgA 1:100)

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

Vers.20160624

Manual

Immuno Fixation Electrophoresis on Biotec-Fischer Agarosegel

for product code
376601-002
376601-102

Intend purpose

The following named reagents are only used to run IFE electrophoresis on agarose gels
The BIOTEC-FISCHER IFX kit enables determination of monoclonal Gammopathien. After the separation of the serum proteins, they can be identified by the precipitation with mono-specific antibodies. For the identification of Gammopathien, antiserum specific for the heavy chains of the immunoglobulin classes and against kappa and lambda light chains may be applied on the electrophoretic patterns of the same serum. The BIOTEC-FISCHER IFX kit makes it possible to determine the antigen specification of the protein as well as the electrophoretic mobility and the quantity of the protein in comparison with other proteins.

Warning

All reagents are for in vitro diagnostic use only. Do not ingest or pipette by mouth any component. Refer to the product safety sheet for component risk and safety phrases in disposal information.
Buffer contains sodiumazide. Do not mix acid solution with sodiumazide to avoid toxic vapors. Always rinse abundantly with water to avoid the formation of metal-azides, because of the potential risk of explosion when piled up in metal conduct-pipes.

Necessary Equipment

Reagents

Prod. No.:	Name
376601	**Kit for 10 IFE on Agarosegel** Content: 1 x 10 agarose gels for 1 tests per gel 3 x 10 thin sheet drying paper for agarose gels 2 x 25 thick gel blotter 1 x 10 sample templates 1 x 10 drying paper for sample templates 1 x 10 antiserum templates 1 x 100 ml buffer concentrate *Fill up 1btl to 1000 ml with aqua dest.* 1 x 50 ml Amido Black staining concentrate *Dilute one bottle with 200 ml Aqua dest.* 1 x 1000 µl Fixing solution for SPE lane 1 x 800 µl IgG Antiserum 1 x 800 µl IgA Antiserum 1 x 800 µl IgM Antiserum 1 x 800 µl Kappa Antiserum 1 x 800 µl Lambda Antiserum

Equipment

Prod. no.:	Name
321001	Electrophoresis tank model 1000
321000	*Electrophoresis tank model BFA (alternat.)*
321101	*Electrophoresis tank FILIPO*
341225	Staining vessel kit for agarose gels
332010	Drying oven KB19
313001	MAESTRO 101 (no further equipment necessary)
331000	Densitometer TurboScan
	Weight of approx. 1-2 kg

Reagents which are not part of kit

Name
Destaining solution *3% Acetic acid (970 ml Aqua dest. + 30 ml iced acid)*
Wash solution *Fill up 9g NaCl with Aqua dest.to 1000 ml total volume*

Shelf live

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

Sample preparation

Freshly collected serum is the specimen of choice.
Sample preparation:
Use fresh or deep-frozen patient sera. Specimens of Plasma are dissuaded because the present of fibrinogen which influences the result. The serum samples are diluted with IFX buffer.

Indication of	Dilution of the serum
Dilution of serum sample for Antisera	1:10 with IFE buffer IgG (10µl + 90µl) 1:5 with buffer IgA/M/K/L (10µl + 40µl)
Dilution SPE lane	1:2 with IFE buffer (10µl + 10µl)
protein concentration	~3.5 mg/ml

Step by step procedure

1. Fill the first vessels with saline solution, the second with staining solution, the next 2 vessels with destaining solution.
2. Fill 35 ml buffer solution in each electrode vessel of migration tank model Filipo (40-45 ml with Beckman - Paragon System)
3. Dry the middle separator with a paper wick if it became wet.
4. Take carefully a gel out of its package, place it flat on the table with gel side up and remove protection shield
5. For gel safety there is a blue protection foil on the gel. Remove it carefully.

Step by step procedure (continued)

6. Take a thin paper wick and place it carefully on the agarose gel to remove the liquid on gels surface. Afterwards remove the paper wick carefully without damaging of lifting off the gel from the support.
7. Place the application template on the marking at cathode (-) side (► ◄)
8. Position the small paper wick on the application slid and press gently to remove air bubbles between template and gel.
9. Fill slid of SP lane with 3 µl diluted serum (one spot in the center of each slid!) and 3 µl to the slid of the Antiserum lanes. **Pay attention for correct dilution!**
10. Wait for 5 minutes and let the samples enter the gel.
11. Position the small paper wick on the application slid to remove the left sample from the template and lift off the template with small paper wick and give it to the waste.
12. Place the gel in to gel bridge with gel side down. **Pay attention for correct position (+ / -)**
13. Place the gel bridge into the tank. If necessary fill up cathode and anode side with buffer until the gel has direct contact with buffer solution
14. Close the lid of migration tank. Take care of correct connection of cathode and anode. (Application side has to be connected to cathode (-) black colored)
15. Run the electrophoresis at 90 Volt for 30 minutes (27 Minutes with Beckman Paragon System @100V)
16. After migration remove the gel from the gel bridge and place it on a table with gel side up.
    IMPORTANT: Gel must not be bended. If gel is bendet take a glass plate larger than gel, give a drop of water onto the glass plate and place the gel support with gel side up on the drop. Adhesive effect keeps gel flat.
17. Take the antiserum template and place it onto the gel aligned with their serum lanes.
18. Make sure that template has complete contact with the gel and there are no air bubbles between gel and template.
19. Take 100 µl of SPE fixation solution and regularly distribute it to the SPE lane. **Pay attention that you do not touch surface of gel with pipette tip.**
20. Take 80 µl of each antiserum and regularly distribute it to the corresponding lane. **Pay attention that you do not touch surface of gel with pipette tip.**
21. Incubate the gel for 20 minutes at 37°C or room temperature
22. After incubation remove the antiserum template carefully from the gel
23. Wash gel for 1 minute in saline solution.