INSTRUCTION MANUAL

SERVA IPG *Blue*Strips

SERVA Electrophoresis GmbH ● Carl-Benz-Str. 7 ● D-69115 Heidelberg
Phone +49-6221-138400, Fax +49-6221-1384010
e-mail: info@serva.de ● http://www.serva.de

Contents

1. SERVA IPG *Blue*Strips **2**

1.1. General information 2

1.2. Scope of supply 2

1.3. Storage conditions 2

2. SERVA IPG *Blue*Strips **3**

3. Rehydration of IPG *Blue*Strips **4**

3.1. Rehydration-/sample buffer 4

4. Sample application **5**

5. Sample concentration **6**

6. Focusing of the IPG *Blue*Strips **6**

6.1. Preparation of focusing in an IEF unit 6

6.2. Focusing conditions 7

6.2.1. Focusing conditions for broad-range IPG gradients pH 3-10, 3-10NL, 6-10 7

6.2.2. Focusing conditions for narrow-range IPG gradients pH 4-7, 3-6, 5-8 7

6.2.3. Conditions for over night focusing 8

6.2.4. Conditions for over day focusing 8

7. Troubleshooting **10**

8. Reagents and equipment for 1D-/2D-gel electrophoresis **17**

9. Literature **17**

1. SERVA IPG *Blue*Strips

1.1. General information

SERVA IPG *Blue*Strips are used in high-resolution 2D-gel electrophoresis and guarantee a stable, reproducible gradient for isoelectrical focusing.
The homogenous acrylamide matrix with immobilized pH-gradient is covalently bound to the support film GEL-FIX™. This stabilizes the gel mechanically and makes the handling easier. Additionally, a non-binding cover film (GEL-FIX for Covers™) protects the gel from damage and contamination.
SERVA IPG *Blue*Strips are dehydrated and have to be rehydrated with a special IEF-2D-sample buffer before use.

The corresponding pH-gradient is printed on the strip. The anodic end is labelled with a „+“. Each gel strip has its own lot number and is therefore suitable for documentation according to GMP/GLP.

All strips of one package are manufactured from a single gel and guarantee highest reproducibility of the results.

1.2. Scope of supply

Packaging size:	12 gel strips SERVA IPG *Blue*Strips
Gel size:	Width 3 mm, gel thickness 0.5 mm
	Length 70 mm, 110 mm, 170 mm, 180 mm, 240 mm
pH gradients:	3 - 10, 3 - 10 NL*, 4 - 7, 6 - 10, 3 - 6, 5 - 8

* NL = non-linear, i.e. in the pH range 5-7 the gradient has a plateau with enhanced resolution

For special gradients please inquire.

1.3. Storage conditions

The recommended storage temperature of the IPG *Blue*Strips is - 20 °C. If stored at the recommended temperature at least usable until: see expiry date on package.

It is important to store the SERVA IPG *Blue*Strips constantly at - 20 °C.

Repeated thawing and freezing cause changes in the gel matrix. As a result, the gel will detach from the supporting film. The detachment may occur during the removal of the cover film or the rehydration step. Therefore, it is essential to avoid repeated freeze-thaw cycles.
Please remove the amount of strips needed for the actual experiment from the package. Store the remaining strips frozen and tightly sealed at - 20 °C.

2. SERVA IPG *Blue*Strips

Cat. No.	pH gradient	length
43001	3-10	7 cm
43002	3-10NL	7 cm
43003	4-7	7 cm
43004	6-10	7 cm
43005	3-6	7 cm
43006	5-8	7 cm
43031	3-10	11 cm
43032	3-10NL	11 cm
43033	4-7	11 cm
43034	6-10	11 cm
43035	3-6	11 cm
43036	5-8	11 cm
43041	3-10	17 cm
43042	3-10NL	17 cm
43043	4-7	17 cm
43044	6-10	17 cm
43045	3-6	17 cm
43046	5-8	17 cm
43011	3-10	18 cm
43012	3-10NL	18 cm
43013	4-7	18 cm
43014	6-10	18 cm
43015	3-6	18 cm
43016	5-8	18 cm
43021	3-10	24 cm
43022	3-10NL	24 cm
43023	4-7	24 cm
43024	6-10	24 cm
43025	3-6	24 cm
43026	5-8	24 cm
43027	3.5-4.5	24 cm

3. Rehydration of IPG *Blue*Strips

3.1. Rehydration-/sample buffer

The optimal buffer composition depends on the applied sample. A sample buffer suitable for many samples is given below in table 1. Use the indicated concentration range of the buffer components as an optimization guideline for difficult samples. Rehydrate the strips either in a special rehydration tray (Cat. No. 43091) or directly in IEF systems for 1D electrophoresis of different manufacturers. In the latter case, please follow the manuals of the manufacturers.

Table 1:

Components	Cat. No.	Concentration range	Amount
Urea *	24524	8 M (8 - 9 M)	4.8 g
CHAPS	17038	1 % (1 – 4 %)	100 mg
DTT	20710	13 mM (13 -100 mM)	20 mg
SERVA HPE™ IPG strip buffer	43368	0.5 % (v/v)	50 µl
Water dest.			ad 10 ml

* can be replaced up to 25 % by thiourea

Note: The sample buffer can be stored in 2.5 ml aliquots at –20 °C for 3 months.

3.2. Rehydration procedure

The necessary volume of rehydration buffer depends on the length of the IPG strip:

Table 2:

Strip length (mm)	Volume/strip (µl)
70	130
110	200
170	320
180	340
240	450

- Pipette the appropriate quantity of rehydration buffer along the edge into the individual recesses of the rehydration tray (approximately in the length of the strip).

- Immerse strip, gel side down (label on the strip legible), at one end into the buffer. Then, by lowering the strip into the buffer, spread the liquid evenly over the whole length of the strip until gel side is ***air bubble-free*** wetted with the solution. ***The strip should float on the liquid and must not adhere to the bottom of the channel.***

- After the solution is completely absorbed by the gel strip (after ca. 5 - 10 min), overlay with 1 - 2 ml SERVA HPE™ IPG Cover Fluid (Cat. No. 43397) to prevent desiccation of the strip.

- Dependent on sample and buffer composition rehydrate for at least 6 hours (preferably over night) ***at room temperature***.

4. Sample application

Two methods exist for application of the sample on the gel strip.
The choice of methods depends on several factors as e.g. sample concentration, sample properties, strip length, pH-gradients and the detection system used (e.g. Coomassie™-, silver staining).

- ***In-Gel* Rehydration**:
  Sample is dissolved in rehydration buffer and the gel strip is incubated in it. Sample volume plus amount of rehydration buffer will give the volume per strip listed in Table 2.

  Advantage:
  - Method for simple sample application.
  - The sample will be highly diluted.

  Disadvantage:
  - Sample application is pH unspecific.
  - Small amounts of sample may be lost in the rehydration tray.

- **Cup Loading**:
  Strip is rehydrated without sample and before IEF the sample is applied to the gel strip with the help of a silicone slot or the like. The location of application depends on used gradient and sample.

  Advantage:
  - Position of sample application is variable.
  - Suitable for low concentrated samples

  Disadvantage:
  - Highly concentrated samples may precipitate.
  - Cup leakage may cause disturbances.
  - Due to slower entering of the sample into the gel matrix the focusing time becomes longer.

5. Sample concentration

The total protein quantity to load per strip has to be individually optimized for each sample.
It depends on the used strip length, gradient and application method as well as the detection system. For silver or SYPRO Ruby staining lower protein quantities are necessary as for staining with Coomassie™ Blue.
To achieve a high resolution, beware of overloading of protein.
In some cases, overloading of protein is acceptable to reveal less abundant proteins of interest.

Recommended range of protein loads for different strip lengths:

IPG *Blue*Strip - 7 cm length: 5 - 300 µg total protein
IPG *Blue*Strip - 11 cm length: 5 - 450 µg total protein
IPG *Blue*Strip - 17 cm length: 50 - 750 µg total protein
IPG *Blue*Strip – 18 cm length: 50 - 750 µg total protein
IPG *Blue*Strip – 24 cm length: 80 - 1000 µg total protein

Note: Do not heat protein samples containing urea above 30 °C to avoid changes in pI values (pI-shift due to protein carbamylation).

6. Focusing of the IPG *Blue*Strips

6.1. Preparation of focusing in an IEF unit

For example: Hoefer IEF 100

- Adjust the cooler to 20 °C.
- Remove the gel strips from the rehydration tray. Place them parallel, with ***the gel side up*** into the focusing tray.
- The end of the strip marked with **+** is oriented towards the anode.
- Wet two electrode wicks slightly with dist. water and position an electrode wick on each gel end.
- When performing Cup-loading, position the cups on the strips and press on firmly.
- To prevent drying of the strips during the focusing cover them with a sufficient amount of SERVA HPE™ IPG Cover Fluid (Cat. No. 43397).
- DIlute sample with rehydartion buffer and bromophenol blue. Depending on strip length the max. volume is 50 -150 µl. Cover samples in each cup with 50 µl of SERVA HPE™ IPG Cover Fluid (Cat. No. 43397).

- Press the electrodes firmly onto the electrode wicks, close the chamber and start focusing.

Note:
The blue stained sample will show every leakage of the cups. Avoid cup leakage because this will lead to loss of sample and distrubances in the 2nd dimension (horizontal stripes on the gel).

6.2. Focusing conditions

Focusing conditions will vary with used strip length, gradient, sample material, and equipment available.
Follow the manufacturer's instructions, if you use special 1D-electrophoresis systems of other suppliers. The following electrophoresis conditions are a guideline for IEF conditions. For optimizing the IEF run, it is possible to change the parameters.

6.2.1. Focusing conditions for broad-range IPG gradients: pH 3-10; 3-10NL; 6-10

Strip length	Initial voltage	Final voltage	Duration
7 cm	150*	3000 V	7 - 8 kVh
11 cm	150*	6000 V	15 - 20 kVh
17 cm / 18 cm	150*	10000V**	30 - 40 kVh
24 cm	150*	12000V**	60 - 80 kVh

6.2.2. Focusing conditions for narrow-range IPG gradients: pH 4-7; 3-6; 5-8

Strip length	Initial voltage	Final voltage	Duration
7 cm	150*	3000 V	8 - 10 kVh
11 cm	150*	6000 V	20 - 35 kVh
17 cm / 18 cm	150*	10000V**	40 - 60 kVh
24 cm	150*	12000V**	60 - 80 kVh

* Hoefer IEF100 minimum adjustable voltage: 250 V
** Some units reach 8000 or 10000 V as maximum voltage.

6.2.3. Conditions for over night focusing

(suitable for cup loading, in gel rehydration and paper bridge sample application)

Temperature : 20 °C					
Current / Strip : up to 70 µA					
Suitable for all IPG gradients [1]					
Strip length			11 cm [2]	17 / 18 cm	24 cm
Step 1	Step	150 V*	3 h	3 h	3 h
2	Step	300 V	3 h	3 h	3 h
3	Gradient	1000 V	6 h	6 h	6 h
4	Gradient	10000 V**	1 h	1 h	1 h
5	Step	12000 V**	1 h	2,5 h	4,5 h
Total time / Duration Vh			14 h / ~ 17 kVh	15.5 h / ~ 32 kVh	17.5 h / ~ 52 kVh

(1) shorten step 5 by 0.5 - 1 h when using basic IPG gradients, e.g. 6-10, 5-11
(2) limit maximum voltage to 6000 V when using 11 cm strips

* Hoefer IEF100 minimum adjustable voltage: 250 V
** Some units reach 8000 or 10000 V as maximum voltage.

6.2.4. Conditions for over day focusing

(suitable for cup loading, in gel rehydration and paper bridge sample application)

Temperature : 20 °C				
Current / Strip : up to 70 µA				
Suitable for all IPG gradients [1]				
Strip length:			7 cm [2]	11 cm
Step 1	Step	150 V*	1 h	1 h
2	Step	300 V	1 h	1 h
3	Gradient	1000 V	1 h	1 h
4	Gradient	3000[2] / 6000 V	2 h	2 h
5	Step	3000[2] / 6000 V	2 h	2,5 h
Total time / Duration Vh			7 h / ~ 10 kVh	7,5 h / ~ 20.5 kVh

(1) shorten step 5 by 0.5 - 1 h when using basic IPG gradients, e.g. 6-10, 5-11
(2) limit maximum voltage to 6000 V when using 11 cm strips

* Hoefer IEF100 minimum adjustable voltage: 250 V
** Some units reach 8000 or 12000 V as maximum voltage.

Note:

- The end point of focusing is determined by the volt-hour-product, so that the samples are always properly focused.
- The focusing time can vary, because the pre-set voltage in the program is not reached immediately every time.
- When using the Multiphor unit, the focusing time has to be prolonged because maximum voltage is only 3000 V.

- For preparative sample application (> 1mg protein per strip) the last focusing step should be prolonged at 15 % of the total time.
- If it is not possible to process the strips immediately after the IEF, it is recommended to switch off the unit and allow the proteins to diffuse in the gel matrix instead of using a low voltage safety step . Before subsequent processing, the proteins on the strips are 15 min focused at maximum voltage.
- If the IPG strips are stained after electrophoresis for control reasons, they have to be fixed in ***20 % trichloroacetic acid*** for ca. 20 min and may be subsequently stained with SERVA Violet 17 staining kit (Cat. No. 35074; for further detail, please visit www.serva.de).

7. Troubleshooting

Symptom	Cause	Remedy
Rehydration solution is distributed unevenly within the gel strip	Some coating in the strip holder or rehydration tray	Wash strip holder and rehydration tray with detergent, rinse with deionized water
	Uneven pipetting of the rehydration solution	Pipette the solution as an even streak
	Rehydration tray or IEF instrument not levelled	Adjust the level of the rehydration tray or the IEF instrument on the bench
Rehydration liquid is left in the rehydration tray or strip holder	Rehydration time too short	Rehydrate at least for 6 hours without and 12 hours with sample
	Liquid volume too high	Follow the recommendations on the package
	IPG strips improperly stored	Always store IPG strips in the freezer, do not leave them on the bench at room temperature for too long time
Basic part of the gel comes off during rehydration	Surface has been damaged during removal of cover film	Always start at the acidic side to remove the cover film
Voltage too low (8 or 10 kV not reached)	Short strips (7 cm and 11 cm) are used, 8 kV is reached in those strips only with some samples under exceptional conditions	Nothing to worry about
	Poor quality of urea and / or thiourea	Use high quality urea and thiourea; remove ions with a mixed bed ion exchanger
	Too much salt in the sample	Remove salts by microdialysis or precipitation; replace PBS for cell washing with something non-ionic like 250 mM sucrose / 1mM Tris
	TCA left in the sample from precipitation	Use 10 % water / 90 % acetone for washing instead of pure acetone

Symptom	Cause	Remedy
Bromophenol blue band stops and does not migrate completely into the anode	Too much salt in the sample	See above
Strip starts to burn at a certain position	Too much salt in the sample	See above
Cover Fluid (paraffin oil) leaks out of the cup loading strip holder during IEF	High protein and salt load cause water transport that carries the oil with it	Reduce the initial voltage and prolong the first low-voltage steps. Or, alternatively use a Manifold (IPGphor), which is more tolerant to high protein and salt concentrations
The basic part of the strip swells during IEF and becomes mechanically instable	Many cations in the sample, for instance Tris	Avoid adding too much Tris-base, replace it by adding 25 mM spermine base, treat the sample with microdialysis; apply IEF strips soaked with deionised water between gel and electrode to accommodate ions
Urea crystallized and IPG strip dried during IEF	Not enough cover fluid used	Use 3 ml IPG Cover Fluid for 18 and 24 cm strip holder and 100 ml for a IPGphor Manifold
	Cover fluid has been moved around in the strip and leaked out	Reduce the initial voltage and prolong the first low-voltage steps
	Running temperature was incorrect	Set the rehydration and separation temperature to 20 °C
Background formed like a “sail” in the basic part of the gel.	Basic carrier ampholytes are not completely removed from the gel during staining	Use only Servalytes® as IPG buffer, because their molecule sizes are smaller than other carrier ampholytes

Symptom	Cause	Remedy
Vertical streaks	Inefficient equilibration a) equilibration steps too short b) volume of equilibration solution too low c) free reducing reagent	a) equilibrate two times for 15 minutes b) use 6 ml for 24 cm strips, 5 ml for 18 cm strips, 3 ml for 11 cm strips, and 2 ml for 7 cm strips c) in 2nd step use alkylation reagent, preferably 2.5 % iodoacetamide
	Silicone oil was used for IPG strip overlay	Use SERVA IPG Cover Fluid for IPG strip rehydration and IEF
No or very few spots	Low protein content	Check with reliable quantification method; check your entire procedure with a standard, like *E.coli* lyophilisate; try alternative sample extraction procedure
	Proteins have formed complexes and did not migrate into the gel	Check sample preparation procedure, apply cleanup based on precipitation
	Problem (s) with silver staining	Check, whether all the solutions have been made correctly, the time for each step has been correct, and no step has been forgotten. Do not use plastic trays, but glass or stainless steel
Missing protein spots in the high molecular weight area	High molecular weight proteins formed aggregates in the first phase of isoelectric focusing	Lower starting voltages over longer time periods
Missing protein spots in the high molecular weight area	Equilibration of IPG strip was too short	Increase equilibration steps to 2 x 20 minutes
	Equilibration of IPG strips was not efficient	Increase SDS concentration in the equilibration buffer to 6 % (w/v)

Symptom	Cause	Remedy
Missing proteins	Sample application on IPG strips was not optimized	Try all alternatives, also cup loading on different sides
	Equilibration was not efficient enough	Increase SDS content in equilibration buffer from 2 to 6 %
Vertical streak and blurred pattern in the acidic area, when thiourea was used in the first dimension	Contaminated reagent	Try another batch of thiourea
	Focusing of thiourea together with some other reagents	Reduce the Volthours applied on the IPG strip. Follow the instructions supplied with the IPG strips, sometimes apply lower Volthours
Vertical gap(s)	Air bubbles between first and second dimension gel	Place the IPG strip carefully on the SDS gel and seal it carefully with agarose
	Amphoteric buffer in the cell culture, for instance HEPES, occupies a part of the pH gradient	Avoid amphoteric buffers
Multiple spots in vertical direction in preparative gels	Insufficient DTT amount	Increase amount of DTT during the first equilibration step
Clouds of spots in the low M_r area	Proteins partly digested into peptides	Add protease inhibitors during protein extraction; use cup loading, paper bridge loading, or active rehydration loading under voltage. Treat the sample with a cleanup procedure based on precipitation
Horizontal streaking	Particles in the sample solution	Always centrifuge the sample before application on the IPG strip. If necessary, prolong centrifugation
	Underfocusing	Prolong the Volthours in the last IEF step

Symptom	Cause	Remedy
Horizontal streaking	Overfocusing: labile proteins are degrading at their pI. This happens particularly in the basic range	Shorten the Volthours in the last IEF step
	Urea and / or detergent concentration too low	Increase concentrations of urea to 9 M and CHAPS to 2 or 4 %
	Wrong precipitation procedure was applied	Do not apply ammonium sulfate precipitation. Either use cleanup kits based on precipitation or apply the procedure according to Wessels and Flügge (methanol / chloroform)
	Incomplete rehydration of the IPG strip	Check the rehydration conditions
	TCA was not completely removed from pellet after precipitation cleanup.	Add two or three washing steps with ice-cold 10 % water / 90 % acetone
	Instability of some proteins because of wrong sample application	Try alternatives, like cup loading at the acidic or basic end of the IPG strip
	Highly abundant proteins have formed ridges and have been squeezed out, and became distributed over the surface	Run IPG strips containing highly abundant proteins only with gel surface up
Horizontal streaking in the acidic area.	Nucleic acids in the sample	Apply a cleanup procedure based on precipitation. Alternatively add Benzonase or DNase and RNase before adding the urea (and thiourea)
Horizontal streaking in the acidic area	DTT depletion in the basic part of the gradient	Pre-rehydrate the strips with hydroxyl ethyl disulfide solution and apply reduced proteins via cup-loading on the anodal side

Symptom	Cause	Remedy
Horizontal streaking in the acidic area	Samples prepared with hydroxyl ethyl disulfide	Never treat the sample with hydroxyl ethyl disulfide. Treat samples with a reductant. Hydroxyl ethyl disulfide should only be used in the IPG strip, not in the sample
	Hydroxyl ethyl disulfide and reductant has been mixed, resulting in the formation of 2-mercaptoethanol	Never mix hydroxyl ethyl disulfide and a reductant, see above.
Horizontal streaking in the acidic area and the basic areas with good separation in the centre	Salt content is too high	Treat the sample with microdialysis or cleanup with precipitation
	Electroendosmosis effects at the ends of narrow interval IPG strips because of accumulated proteins of higher and lower pIs	Place filter paper pads soaked in deionised water between electrodes and gel during the last IEF step
Horizontal streaking in the centre with good separation in the acidic area and the basic areas	Uneven rehydration of the IPG strip, incomplete rehydration in the middle	During rehydration of the strip take care, that the liquid is distributed evenly over the entire strip length. Alternatively, use a vertical rehydration cassette for rehydration of IPG strips
Protein spots arranged like a string of beads in horizontal direction	Differential carbamylation of some proteins because of presence of isocyanate	Do not heat sample. Do not store urea and thiourea solutions at room temperature. Use only high quality urea and thiourea. Remove ionic compounds with a mixed bed ion exchanger
	Storage proteins in plant seed extracts, differentially glycosylated	No artifact. Nothing to worry about
Cloudy background and/or front	Micelles between SDS and the nonionic or zwitterionic detergent have formed	Reduce content of CHAPS or alternative detergent in the rehydration solution for the IPG strip (e.g. maximum 2 % CHAPS)

Symptom	Cause	Remedy
Blurred and streaky spot pattern in preparative gels	Insufficient IEF conditions	Prolong the volthours in the last IEF step by 15 %.
	DTT depletion in preparative basic gels	Add 2.5 % DTT, 20 % isopropanol, 5 % glycerol to the rehydration solution, apply paper wick soaked in rehydration solution plus 3.5 % DTT at the cathode. Or apply hydroxyl ethyl disulfide
Blurred spot pattern in ready-made gels	Liquid and / or bubbles between surface of ready-made gel and glass plate	Use only low amount of gel buffer and roll out excess buffer and air bubbles completely
Very basic proteins are lost	Too many Volthours applied on basic gradients, autohydrolysis of basic buffering groups in the gel	Shorten the Volthours in the last IEF step
"Wrong" proteins coming from other organisms, which should not be present, have been identified in the gel	Contamination with previous sample or proteins from the laboratory environment	Use only highly pure reagents; clean equipment thoroughly, particularly rehydration tray and strip holders for IPG strips; filter solutions through a membrane filter
No protein focusing at all.	Rehydration-/sample buffer saturated with CO_2	Do not use dry ice for cooling/freezing rehydration-/sample buffer

8. Reagents and equipment for 1D-/2D-gel electrophoresis

Product	**Cat. No.**
SERVA HPE™ IPG Cover Fluid	43397
Urea	24524
Dithiothreitol (DTT)	20710
CHAPS	17038
SERVA HPE™ IPG strip buffer	43368
SERVA Proteome Markers	39220
Trichloroacetic acid, 20 % solution	36913
Triton® X-100	39795
Glycerol from plant	23176
SDS, 20 % solution	39575
Bromophenol Blue-Na-salt	15375
Iodoacetamide	26710
Acrylamid/Bis Solution 37.5:1 (40 % w/v), 2.6 % C	10681
N,N,N',N'-Tetramethyl-ethylenediamine (TEMED)	35925
Ammonium persulfate	13375
Laemmli Buffer 10X, for SDS-PAGE	42556
Rehydration tray for IPG ***Blue***Strips	43091
BlueHorizon™ Super Cool Flatbed System	BH-2C
BluePower 3000 power supply	BP-3000
Circulatory Refrigerator Bath WK 230	WK230

9. Literature

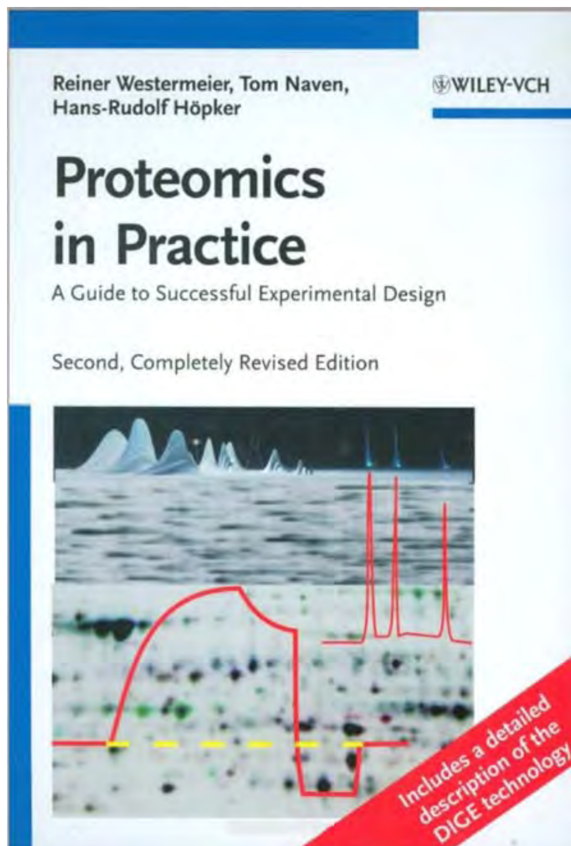
- The Current State of Two-dimensional Electrophoresis with Immobilized pH Gradients,
  A. Görg, C. Obermaier, G. Boguth, A. Harder, B. Scheibe, R. Wildgruber, W. Weiss, *Electrophoresis* 2000 Apr;21(6):1037-53

- Altland, K., *Electrophoresis* 1986, 7, 251-259

- Görg, A., Boguth, G., Obermaier, C., Weiss, W., *Electrophoresis* 1998, *19*, 1516-1519

- Altland, K., *Electrophoresis* 1990, *11*, 140-147

Further electrophoresis and proteomics literature:



Westermeier R, Naven T, Hoepker HR.
Proteomics in Practice. 2nd ed.
WILEY-VCH, Weinheim (2008).
ISBN 978-3-527-31941
(Directly obtainable by SERVA Electrophoresis)