

INSTRUCTION MANUAL

SERVA Ge™ Neutral pH 7.4 **SERVA Ge™ Neutral pH 7.4 Gradient** Precast Vertical Gels for Electrophoresis

(Cat. Nos. 43220.01, 43220.02, 43220.03, 43221.01, 43221.02, 43221.03)



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Contents

1. SERVA[™]Ge Neutral pH 7.4 and SERVA[™]Ge Neutral pH 7.4 Gradient	2
1.1. General information	2
1.2. Scope of supply and product description	2
1.3. Composition of gels	3
1.4. Storage conditions	3
2. Handling of gel cassettes/electrophoresis procedure	3
3. Electrophoresis protocol	4
3.1. Separation range of gels	4
3.2. Running buffer preparation	4
3.2.1. Laemmli buffer for SDS PAGE	4
3.2.2. Tris-Tricine/SDS running buffer	4
3.2.3. Tris-MOPS/SDS running buffer	4
3.3. Sample preparation	5
3.3.1. Denaturing conditions (SDS)	5
3.3.2. Recommended sample quantity	5
3.4. Electrophoresis conditions	5
3.4.1. Electrophoresis with Laemmli buffer	5
3.4.2. Electrophoresis with Tris-Tricine/SDS running buffer	6
3.4.3. Electrophoresis with Tris-MOPS/SDS running buffer	6
4. Staining	6
4.1. Staining with SERVA Blue R	6
4.1.1. Reagents and solutions	6
4.1.2. Protocol	7
5. Protein transfer	7
5.1. Tank blotting	7
5.2. Semi-dry blotting	8
6. Trouble shooting	9
7. Order information	10

1. SERVA[™]Ge Neutral pH 7.4 and SERVA[™]Ge Neutral pH 7.4

1.1. General information

SERVA[™]Ge Neutral pH 7.4 gels are ready-to-use vertical gels with a neutral buffer system, which allows optimal separation of small and large proteins in one gel.

Benefits of the product for the user:

- simple, fast handling
- high resolution, sharp bands, best reproducibility
- made from top-quality chemicals
- gels prepared in unbreakable plastic cassette, leakage-free
- long separation distance, cm-scale at front of cassette allows reproducible runs
- marking of anode and cathode for error-free assignment
- extra tool provided for easy and safe opening of cassette at the end of run
- compatible with many commercially available electrophoresis tanks (e.g. SERVA BlueVertical 102, Hoefer Mighty Small[™] SE 260, Hoefer miniVE[™], NOVEX XCell II[®], etc.)

The precast gels are manufactured according to proprietary methods developed by SERVA Electrophoresis GmbH and are subject to strict quality control. Each production batch has assigned a unique lot number. In the event of queries, please quote this lot number along with the catalogue number.

1.2. Scope of supply and product description

Packaging size:

Cat. No. 43220.01	Box with 10 neutral gels
Cat. No. 43220.02	Box with 6 neutral gels
Cat. No. 43220.03	Box with 2 neutral gels
Cat. No. 43221.01	Box with 10 neutral gels
Cat. No. 43221.02	Box with 6 neutral gels
Cat. No. 43221.03	Box with 2 neutral gels

Gels are packed individually and vacuum-sealed Each gel is packed individually sealed in an aluthene bag. It is protected from desiccation by a layer of filter paper moistened with gel buffer. Each box contains a tool for opening of cassette.

Cassette:	Outer dimensions	10 cm x 10 cm
	Number of sample wells	12
	Volume of well	35 µl
Gel:	Material:	Arylamide/N, N'-methylene bisacrylamide
	Dimensions separation gel	Length 7 cm x width 8 cm
	Thickness of gel layer	1 mm

1.3. Composition of gels

SERVAGe™ Neutral pH 7.4 gels contain **no SDS**. The separation range of the gels for denatured proteins is shown in table 3.1. (page 18).

Acrylamide concentration (T):	8 %, gradient
Cross linker concentration (C):	2.6 %
Stacking gel:	5 % T, 2.6 % C
Gel buffer:	neutral buffer system pH 7.4

1.4. Storage conditions

Store the gels at 2 – 8 °C. Do **not** freeze the gels or leave them at room temperature for longer periods as this may impair their separation properties. If stored at the recommended temperature at least usable until: see expiry date on package.

2. Handling of gel cassettes/electrophoresis procedure

Safety information:

For safety reasons always wear suitable protective gloves and clothing, when you work with gels and appending solutions.

1. Remove gels from cardboard box. If only one gel is required, immediately place the remaining gels again to storage at 2 – 8 °C. Cut open aluthene bag along the upper edge using scissors. Remove gel.
2. Place the gel into the electrophoresis chamber so that the opened (“u-shaped”) side of the cassette is facing towards the cathode buffer tank. Follow the manual of your electrophoresis chamber supplier for detailed instructions.
3. Add the electrophoresis buffer. Pull the comb steadily out of the gel; remove eventually remaining gel rests above the sample wells. Rinse the sample wells thoroughly, avoiding and/or removing any air bubbles.
4. Apply samples. Load those sample wells without samples with sample buffer (1x).
5. Close the electrophoresis chamber and connect to power supply. Switch on power supply and begin electrophoresis.
Conditions: see paragraph 3.
6. On completion of electrophoresis, switch off power supply, disconnect the electrophoresis chamber, remove electrophoresis buffer and remove cassettes.
7. To open cassette hold cassette upright with its bottom end supported by a table or bench. Place the corner of the key marked by an arrow at the upper right-hand end of the grooved edge of the cassette (also marked by an arrow) and break open the cassette with a swift blow from above on the key. Turn around the cassette and open the other side in the same way.
8. To remove the gel, carefully detach the plates so that the gel remains on one.
Gels can now be stained or used for blotting.

3. Electrophoresis protocol

3.1. Separation range of gels

Acrylamide concentration (%)	Separation range (Mr 10 ³)
8	6,5- 200
Gradient	6,5 - 200

3.2. Running buffer preparation

3.2.1. Laemmli buffer for SDS PAGE

Dilute 10x Laemmli buffer for SDS PAGE 1:10 (Cat. No. 42556; composition see table below), pH value 8.8.

Components	Concentration	Amount
Tris	0.25 M	30 g/l
Glycine	1.92 M	144 g/l
SDS	1 %	10 g/l

3.2.2. Tris-Tricine/SDS running buffer

Dilute 20x Tris-Tricine/SDS running buffer 1:20 (composition see table below), pH value 8.5.

Components	Concentration	Amount
Tris	1.2 M	145 g/l
Tricine	0.8 M	143 g/l
SDS	2 %	20 g/l

3.2.3. Tris-MOPS/SDS running buffer

Dilute 20x Tris-MOPS/SDS running buffer 1:20 (composition see table below), pH value 8.4.

Components	Concentration	Amount
Tris	1.2 M	145 g/l
MOPS	0.6 M	126 g/l
SDS	2 %	20 g/l

3.3. Sample preparation

3.3.1. Denaturing conditions (SDS)

SERVA Tris/Glycine/SDS sample buffer (2x), Cat. No. 42527, does not contain any reduction reagent. By adding 5 % 2-mercaptoethanol (Cat. No. 28625) or 10 mM DTT (Cat. No. 20710) you determine whether or not reducing conditions prevail (concentrations refer to 1x sample buffer). Since the reduction reagents oxidise in time, the buffer should always be **freshly** prepared. The samples are diluted (1:1) with an equal volume of 2x sample buffer and mixed well (denaturing, composition for sample buffer for self-preparation see table). The maximal well volume is 35 µl.

- Heat sample for 5 minutes at 95 °C. Heat fluorescence-labelled sample for 5 minutes at 65 °C.
- Rinse wells with running buffer.
- Load samples and start electrophoresis.

Sample buffer components	Concentration 2x sample buffer	Amount
1 M Tris-HCl pH 6.8	0.126 M	0.625 ml
10 % (w/v) SDS	4 %	2 ml
Glycerol	20 %	1 ml
0.1 % (w/v) Bromophenol blue	0.02 %	0.5 ml
2 M DTT	0.2 M	0.5 ml
<i>Or: 2-Mercaptoethanol</i>	<i>10 %</i>	<i>0.25 ml</i>
Water, deion.		ad 5 ml

3.3.2. Recommended sample quantity

Amount/band	Staining method	SERVA product
0.1 – 0.5 µg protein	SERVA Blue, Coomassie [®] Brilliant Blue	DensiStain BlueG Soln., SERVA Blue R Tablet Staining Kit
10 - 50 ng protein	Silver staining	Silver Staining Kit SDS PAGE

3.4. Electrophoresis conditions

Electrophoresis is carried out under the following conditions:

3.4.1. Electrophoresis with Laemmli buffer

Let samples run into the gel for 15 minutes at 10 mA/gel.
Adjust then a limiting amperage of 20 mA/gel.
Voltage will raise during the run from initial ca. 60 V to 250 V.

Duration: ca. 70 min.

We recommend running the gel at constant amperage.

Alternatively run can be performed at constant voltage of 150 V. Amperage falls during run from initial 20 - 25 mA to ca. 10 mA. **Duration:** ca. 90 min.

3.4.2. Electrophoresis with Tris-Tricine/SDS running buffer

Gels are run at constant voltage of 150 V. Amperage falls during run from initial ca. 35 mA to ca. 20 mA.

Duration: ca. 50 min.

Alternatively run can be performed at constant voltage of 200 V. Amperage falls during run from initial 50 - 60 mA to 20 - 30 mA.

Duration: ca. 30 min.

Important: At these conditions gels must be sufficiently cooled to prevent “smile effects”.

3.5.3. Electrophoresis with Tris-MOPS/SDS running buffer

Gels are run at constant voltage of 150 V. Amperage falls during run from initial ca. 35 mA to ca. 20 mA.

Duration: ca. 50 min.

Alternatively run can be performed at constant voltage of 200 V. Amperage falls during run from initial 50 - 60 mA to 20 - 30 mA. Duration: ca. 30 min.

Important: At these conditions gels must be sufficiently cooled to prevent “smile effects”.

4. Staining

Safety information:

For safety reasons, always wear protective gloves and clothing, when working with fixing and staining solutions.

For best results use user-friendly staining kits from SERVA like SERVA *DensiStain* Blue G Staining Solution (Cat. No. 35078.01), SERVA Blue R Tablet Staining Kit (Cat. No. 35079.01) or SERVA Silver Staining Kit SDS PAGE (Cat. No. 35076.01) resp. for native gels SERVA Silver Staining Kit Native PAGE (Cat. No. 35077.01).

You can also use other common staining protocols as e.g. the protocol described in paragraph 4.1:

4.1. Staining with SERVA Blue R

4.1.1. Reagents and solutions

Stock solution 1	0.2 % SERVA Blue R in 90 % (v/v) ethanol (Cat. No. 11093) (Solve 100 mg SERVA Blue R (Cat. No. 35051) in 50 ml ethanol)
Stock solution 2	20 % (v/v) acetic acid
Destainer	20 % (v/v) ethanol, 5 % (v/v) acetic acid, 1 % (w/v) glycerol (Kat.-Nr. 23176)
Preservation solution	30 % (v/v) ethanol, 5 % (w/v) glycerol

4.1.2 Protocol

Carry out all fixing and staining work on a shaker at moderate speed (50 rev/min). The specified times apply to incubation at room temperature. Shorter staining and destaining times can be achieved by increasing the temperature.

Fixation/staining	Fixation and staining are done in step. Stock solution 1 and 2 are mixed in equal parts and the gel is incubated for 30 min. in the solution. (Staining solution can be re-used for 2 - 3 xs.)
Destainer	Rinse gel after staining for 1 minute with dest. water and incubate for 2 x 60 minutes in destainer. If background is not clear enough, destain gel for 20 - 30 minutes in 40 % ethanol/10 % acetic acid/2 % glycerol.
Preservation	Incubate gel over night in preservation solution. The gel can then be dried in a drying frame.

5. Protein transfer

Safety information:

For safety reasons, always wear protective gloves and clothing, when working with gels and buffer solutions.

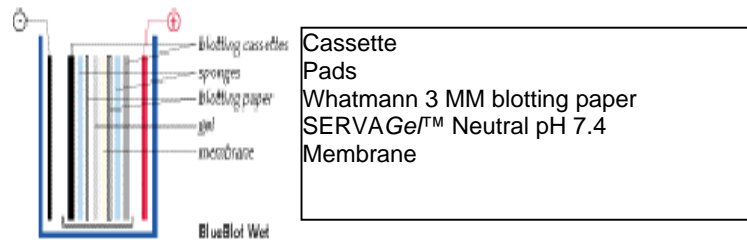
Blotting of SERVAGe™ Neutral pH 7.4 gels can be done in tank blotter or in semi-dry blot systems. Thereby continuous and discontinuous buffer systems can be used.

Note: Please comply with the instructions of the manufacturer of the blotting apparatus regarding to transfer parameter and time (in particular to the data referring to max. amperage and max. voltage of the blotting device). Transfer time is dependent on size and charge of sample proteins and must be optimized for each sample. For marker proteins of middle molecular sizes a transfer time of 60 min. is sufficient.

5.1. Tank blotting

1. Cut transfer membrane and four pieces Whatmann 3 MM paper to gel size (7 x 8 cm).
2. Equilibrate the membrane in transfer buffer (Towbin buffer, Cat. No. 42558). By use of PVDF membranes equilibrate first for 2 minutes in methanol and then for additional 5 minutes in transfer buffer.
3. Wet the porous pads as well as the four pieces Whatmann 3 MM paper with transfer buffer.
4. Remove the gel from the cassette (s. p. 18) and equilibrate it for 5 minutes in transfer buffer.

5. Mount the transfer sandwich and place it in the tank blotter.



6. Transfer is done at room temperature at 250 mA resp. ca. 60 V for ca. 1 – 2 hours (for standard marker proteins).

5.2. Semi-Dry blotting

1. Cut transfer membrane and four pieces Whatmann 3 MM paper to gel size (7 x 8 cm).
2. Equilibrate the membrane in transfer buffer (Towbin buffer, Cat. No. 42558). By use of PVDF membranes equilibrate first for 2 minutes in methanol and then for additional 5 minutes in transfer buffer.
3. Wet the porous pads as well as the four pieces Whatmann 3 MM paper with transfer buffer.
4. Remove the gel from the cassette (s. p. 18) and equilibrate the gel for 5 minutes in transfer buffer.
5. Mount the transfer sandwich analogue to the tank blot sandwich and place it into the semi-dry blotter.
6. Transfer is done at room temperature with 1.5 mA/cm² gel area for ca. 1 hour (for standard marker proteins).

By transfer of differently large proteins the use of a discontinuous blotting buffer system is recommended (SERVA Semi-Dry blotting kit, Cat. No. 42559.01)

After blotting proteins can be stained on the membrane:

- **Detection with Ponceau S solution** (0.2 %, Cat. No. 33427): Overlay the washed membrane with ready-to-use Ponceau S solution and stain for ca. 5 min. with moderate shaking. Destain background with H₂O dest. until the red bands are clearly visible.
- **Staining with Amido black:** Incubate membrane for 5 minutes in Amido black staining solution (dilute 1 % Amido black in 40 % ethanol and 10 % glacial acetic acid 1:10), then destain in destaining solution (40 % ethanol, 10 % glacial acetic acid and 2 % glycerol).

Note: Amido black is no reversible staining, however more sensitive as Ponceau S, comparable with Coomassie Brilliant Blue R staining.

4. Trouble shooting

Problem	Possible cause	Countermeasure
No current	Unclosed circuit	Check contacts/leads at source of current and separation chamber; check buffer level
Low current	Wrong adjustment of parameters at power source	For limiting amperage select the maximum voltage recommended for the chamber; for limiting voltage select maximum amperage
'Smile effect' at buffer front	Overheating	Pre-cool buffer; cooling via cooling circulator or a reduction in amperage
Slow migration of buffer front	Running buffer fully consumed	Always use fresh running buffer
Blurred bands	Diffusion after application of samples	Apply samples quickly; begin electrophoresis straight away
	Diffusion after separation	Transfer gel to fixing or staining solution immediately after electrophoresis
Irregular bands	Sample volumes too low or too different	Apply at least 5 µl sample; use approx. the same amounts of sample
	Differing saline content of samples	Desalinate samples as required (dialysis, gel filtration)
Formation of stripes	Precipitation of sample	Centrifuge or filter sample
Wide, partially smeared bands	Lipophilic substances in the sample	Remove substances prior to electrophoresis; increase SDS concentration if necessary
More bands than expected	Protease activity	Add protease inhibitor; minimise time between sample preparation and run
	Incomplete reduction	Check reduction conditions (if necessary prolong incubation time; increase DTT concentration)

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XCell II® and ThermoFlow® Mini-Cell are trademarks of Novel Experimental Technology.

Coomassie® is a trademark of ICI Ltd.

5. Order information

Precast gels	Cat No.
SERVAGel™ Neutral pH 7.4 (10 gels)	43220.01
SERVAGel™ Neutral pH 7.4 (6 gels)	43220.02
SERVAGel™ Neutral pH 7.4 (2 gels)	43220.03
SERVAGel™ Neutral pH 7.4 Gradient (10 gels)	43221.01
SERVAGel™ Neutral pH 7.4 Gradient (6 gels)	43221.02
SERVAGel™ Neutral pH 7.4 Gradient (2 gels)	43221.03
Equipment	
BlueVertical mini slab gel system BV 102	BV 102
Blue Power 500 Plus power supply	BP-500Plus
BlueBlot Wet 100 tank blotter (10 x 10 cm)	BB 100
BlueFlash Semi-Dry blotter medium (15 x 15 cm)	BF-M
Protein marker	
SERVA Protein Test Mixture 6 for SDS PAGE (6.5 – 94.7 kDa)	39207.01
SERVA Unstained SDS PAGE Protein Marker (6 – 200 kDa)	39215.01
SERVA Prestained SDS PAGE Protein Marker (6 – 200 kDa)	39216.01
SERVA Recombinant SDS PAGE Protein Marker (10 – 150 kDa)	39217.01
SERVA Recombinant SDS PAGE Protein Marker PLUS (10 – 150 kDa)	39218.01
Protein MW Standards for Native PAGE (12 – 450 kDa)	39064.01
Staining reagents and kits:	
SERVA DensStain Blue G Staining Solution (2x concentrated, 500 ml)	35078.01
SERVA Blue R Tablet Staining Kit (2 x 500 ml)	35079.01
SERVA Silver Staining Kit SDS PAGE (25 mini gels)	35076.01
SERVA Silver Staining Kit Native PAGE (25 mini gels)	35077.01
SERVA Blue G	35050
SERVA Blue R	35051
Amido black10 B (50 g)	12310.01
Ponceau S solution (0.2 %, 500 ml)	33427.01
Silver nitrate	35110
Buffers etc.	
Laemmli buffer 10x, for SDS PAGE	42556
Towbin buffer 10x, for native PAGE and for Western Blotting (1 L)	42558.01
Semi-Dry blotting buffer kit (3 x 500 ml)	42559.01
Bromophenol blue, sodium salt	15375
Dithiothreitol	20710
Ethanol, undenatured, absolute	11093
Glycerol (1L)	23176.01
2-Mercaptoethanol	28625
Glycine	23390
Morpholinopropane sulfonic acid (MOPS)	29836
N-Tris(hydroxymethyl)methylglycine (Tricine)	37195
Tris(hydroxymethyl)aminomethane	37186
SDS in Pellets	20765
SDS solution, 20 % (w/v)	20767
Membranes	
Immobilin (PVDF), 9 x 12 cm, pore size: 0.2 µm (10 sheets)	42579.01
Immobilin (PVDF), 26.5 cm x 3.75 m, pore size: 0.2 µm (1 roll)	42574.01
Fluorobind (PVDF), 25 cm x 3 m, pore size: 0.2 µm (1 roll)	42571.01
Fluorobind (PVDF), 10 x 10 cm, pore size: 0.2 µm (20 sheets)	42573.01