INSTRUCTION MANUAL

SERVA*Gel*™ Neutral HSE

Precast Vertical Gels for Electrophoresis

(Cat. No. 43245, 43246)

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

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Vers. 03/12

1. SERVA*Gel*™ Neutral HSE

1.1. General information

SERVA*Gel*™ Neutral HSE gels are ready-to-use vertical gels with a neutral buffer system, which allows optimal separation of small and large proteins in one gel.

These gels are compatible with SDS-Tris-Glycine (Laemmli) as well as SDS-Tris-Tricine buffer system in standard electrophoresis.

Additionally, SERVA*Gel*™ Neutral HSE gels can be used at 400 V with the SDS-Tris-Glycine buffer system (Laemmli). The electrophoresis time is reduced to ca. 20 min. To get optimal results and efficient cooling of the gel, the capacity of the buffer tank should be large enough.

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™ PRiME™, Hoefer Mighty Small™ SE 260, Hoefer miniVE™SE 300, 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. 43245.01, 43246.01 Box with 10 gradient neutral gels
Cat. No. 43245.03, 43246.03 Box with 2 gradient neutral gels

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	10 or 12
Volume of well	50 µl for 10-well gels and 35 µl for 12-well gels

Gel:

Material	Acrylamide/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

SERVA*Gel*™ Neutral HSE gels contain **no SDS**. The separation range of the gels for denatured proteins is shown in table 3.1.

Acrylamide concentration (T): gradient 4-12 %

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. Standard electrophoresis protocol

3.1. Separation range of gels

Acrylamide concentration (%)	Separation range (Mr 10^3)
4-12 %	6.5 - 200

Gradient gels give sharper band resolution in the separation range of ca. 5 - 100 kDa.

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 8.6.

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 (Cat. No. 42560.01, 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

3.3. 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 (12 wells) and 50 µl (10 wells).

- 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 %	1 ml
2 M DTT	0.02 M	0.05 ml
Or: 2-Mercaptoethanol	*10 %*	*0.5 ml*
Water, deion.		ad 5 ml

By usage of a **Tris-Tricine running buffer** it is recommended to employ a Tris-Tricine/SDS sample buffer:

SERVA Tris-Tricine/SDS sample buffer (2x), Cat.-No. 42551.01:
900 mM Tris/HCl (pH 8.45), 24 % Glycerol, 4 % SDS, 0.015 % SERVA Blue G and 0.005 % Phenol red.

3.3.2. Recommended sample quantity

Amount/band	Staining method	SERVA product
0.1 – 0.5 µg protein	SERVA Blue, Coomassie® Brilliant Blue	*Densi*Stain BlueG Solution, SERVA Blue R 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 limiting amperage of 20 mA/gel.
Voltage will increase 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".

4. High speed electrophoresis

This protocol will reduce the duration of the electrophoresis to 20 min.

IMPORTANT: To perform an electrophoresis according to this protocol, please use only the SDS-Tris-Gylcine (Laemmli) buffer system. Please note also, to get optimal results and efficient cooling of the gel, the capacity of the buffer tank should be large enough, e.g. SERVA BlueVertical 102, Hoefer miniVE™SE 300, NOVEX XCell II® (for details see section 4.4.).

4.1. Sample preparation

- Dilute the samples with an equal volume of 2x sample buffer and mix well (denaturing, composition for sample buffer for self-preparation see table below). The maximal volume per well is 35 µl (12 wells) and 50 µl (10 wells).

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 %	1 ml
2 M DTT	0.02 M	0.05 ml
Or: 2-Mercaptoethanol	*10 %*	*0.5 ml*
Water, deion.		ad 5 ml

- Heat sample for 5 minutes at 95 °C. Heat fluorescence-labelled sample for 5 minutes at 65 °C.

4.2. Running Buffer Preparation

To prepare 1x Tris-Glycine/SDS Electrophoresis Buffer mix 100 ml of 10x stock solution (SERVA Cat. No. 42556) and 900 ml H_2O bidest.

Components	Concentration (10x buffer)	Amount
Tris	0.25 M	30 g/l
Gylcine	1.92 M	144 g/l
SDS	1 %	10 g/l

4.3. Sample application

- Rinse wells with running buffer.
- Load samples (max. sample volume per well: 35 µl for 12-well gels and 50 µl for 10-well gels)

Recommended sample quantity

Amount/band	Staining method	SERVA product
0.1 – 0.5 µg protein	SERVA Blue, Coomassie® Brilliant Blue	*Densi*Stain Blue G Solution SERVA Blue R Staining Kit
10 - 50 ng protein	Silver staining	Silver Staining Kit SDS PAGE

4.4. Filling the buffer chamber

Use 1x running buffer for the electrophoresis. The buffer level of the outer buffer chamber should at least reach the lower end of the sample wells to optimize the cooling of the gel.

4.5. Running conditions

	1 gel per unit	
Voltage	400 V	300 V
Amperage	approx. 105 mA (Start); 65 mA (End)	approx. 70 mA (Start); 35 mA (End)
Time	approx. 20 min	approx. 30 min

Please note: During electrophoresis the running buffer becomes extremely warm. Therefore only one gel per unit should be used. If **2 gels** are used, it is recommended to **reduce the voltage to 200 V**. The **time** for the electrophoresis will then be **doubled**.

4.6. Gel staining

For staining common staining protocols are suitable, e.g. SERVA Blue R (Cat. No. 42531) (see section 5.).

5. 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 *Densi*Stain Blue G Staining Solution (Cat. No. 35078.01), SERVA Blue R Staining Kit (Cat. No. 42531.01) 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 section 5.1:

5.1. Staining with SERVA Blue R

5.1.1. 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 (Cat. No. 23176)
Preservation solution	30 % (v/v) ethanol, 5 % (w/v) glycerol

5.1.2. 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 one 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.

6. Protein transfer

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

Blotting of SERVA*Gel*™ Neutral HSE 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.

6.1. Tank blotting

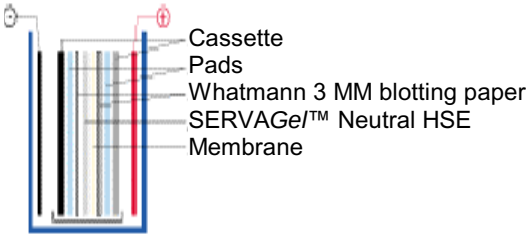
1. Cut transfer membrane and four pieces Whatmann 3 MM paper to gel size (7 x 8 cm).

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 (see chapter 2) 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).

6.2. 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 (see chapter 2) 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/cm^2 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 H_2O 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.

7. 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 or distorted 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)
	Overheating of the gel due to insufficient coolling when using the high speed protocol	Use adequate volume of buffer in the outer buffer chamber (see section 4.4.)
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)

8. Order information

Precast Gels	**Cat.No.**
SERVA*Gel*™ Neutral HSE, 12 sample wells	43245
SERVA*Gel*™ Neutral HSE, 10 sample wells	43246
SERVA*Gel*™ Neutral HSE, 2D	43247
SERVA*Gel*™ Neutral HSE Starter Kit	43207
SERVA*Gel*™ Neutral pH 7.4, 12 sample wells	43220
SERVA*Gel*™ Neutral pH 7.4, 10 sample wells	43222
SERVA*Gel*™ Neutral pH 7.4 Gradient, 12 sample wells	43221
SERVA*Gel*™ Neutral pH 7.4 Gradient, 10 sample wells	43223
SERVA*Gel*™ PRiME™ 8, 12 sample wells	43260
SERVA*Gel*™ PRiME™ 8, 10 sample wells	43261
SERVA*Gel*™ PRiME™ 10, 12 sample wells	43263
SERVA*Gel*™ PRiME™ 10, 10 sample wells	43264
SERVA*Gel*™ PRiME™ 12, 12 sample wells	43266
SERVA*Gel*™ PRiME™ 12, 10 sample wells	43267
SERVA*Gel*™ PRiME™ 12, 2D sample well	43268
SERVA*Gel*™ PRiME™ 14, 12 sample wells	43269
SERVA*Gel*™ PRiME™ 14, 10 sample wells	43270
SERVA*Gel*™ PRiME™ 14, 2D sample well	43271
SERVA*Gel*™ PRiME™ 4-12, 12 sample wells	43273
SERVA*Gel*™ PRiME™ 4-12, 10 sample wells	43274
SERVA*Gel*™ PRiME™ 4-20, 12 sample wells	43276
SERVA*Gel*™ PRiME™ 4-20, 10 sample wells	43277
SERVA*Gel*™ PRiME™ 8-16, 12 sample wells	43279
SERVA*Gel*™ PRiME™ 8-16, 10 sample wells	43280
SERVA*Gel*™ PRiME™ Starter Kit	43206
Equipment	
BlueVertical PRiME™ Mini Slab Gel System	BV104
Blue Power 500x4 Power Supply	BP-500x4
BlueFlash Semi-Dry Blotter Medium (15 x 15 cm)	BF-M
Protein Marker	
SERVA Protein Test Mixture 6 for SDS PAGE (6.5 – 97.4 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 *Densi*Stain Blue G Staining Solution (2fach konzentriert, 500 ml)	35078.01
SERVA Blue R Staining Kit (2 x 500 ml)	42531.01
SERVA Silver Staining Kit SDS PAGE (25 Minigele)	35076.01
SERVA Silver Staining Kit Native PAGE (25 Minigele)	35077.01
SERVA Blue G	35050
SERVA Blue R	35051
Amidoschwarz 10 B (50 g)	12310.01
Ponceau S Lösung (0,2 %, 500 ml)	33427.01
Silbernitrat	35110

Buffers etc.	
SERVA Tris-Glycine/SDS electrophoresis buffer (10x)	42529
SERVA Tris-Glycine/SDS sample buffer (2x)	42527
SERVA Tris-Glycine native electrophoresis buffer (10x)	42530
SERVA Tris-Glycine native sample buffer (2x)	42528
Laemmli Buffer 10x, for SDS PAGE	42556
SERVA Tris-Tricine/SDS electrophoresis buffer (20x)	42560
SERVA Tris-Tricine/SDS sample buffer (2x)	42551
Towbin Buffer 10x, for Western Blotting (1 L)	42558.01
Semi-Dry Blotting Buffer Kit (3 x 500 ml)	42559.01
Bromphenolblau, Natriumsalz	15375
Dithiothreitol	20710
Ethanol, undenaturiert, absolut	11093
Glycerin (1L)	23176.01
2-Mercaptoethanol	28625
Glycine	23390
N-Tris(hydroxylmethyl)methylglycine (Tricine)	37195
Tris(hydroxymethyl)aminomethan	37186
SDS in pellets	20765
SDS solution, 20 %	20767

Membranes	
Immobilon (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