INSTRUCTION MANUAL

SERVA*Gel*™ Neutral HSE 2D

Precast Vertical Gels for Electrophoresis

(Cat. No. 43247)

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Vers. 03/12

1. SERVA*Gel*™ Neutral HSE 2D

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.

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™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. 43247.01	Box with 10 gradient neutral gels
Cat. No. 43247.03	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	one 2D well

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. Electrophoresis

The second dimension of 2D gel electrophoresis involves reducing and alkylating the proteins focused on your IPG strip in equilibration buffer, loading the strip in your second dimension gel, and performing SDS-PAGE.
Safety information:
For safety reasons always wear suitable protective gloves and clothing, when you work with gels, IPG strips and appending solutions.

3.1. Equilibrating the IPG strip

3.1.1. Reagents and solutions

Equilibration solution (50 mM Tris/HCl pH 8.8, 6M urea, 30 % glycerol, 2 % SDS, 0.01 % Bromphenolblau)

Components	Concentration	Amount (to 100 ml)
1.5 M Tris/HCl pH 8.8	0.05 M	3.3 ml
Urea (cat. no. 24524)	6.0 M	36 g
100 % Glycerol (cat. no. 23176)	30 %	30 g
20 % SDS solution (cat. no. 20767)	2 %	10 ml
Bromophenol blue Na-salt (cat. no. 15375)	0.01 %	10 mg
H_2O dest.		ad 100 ml

Dispense solution in 10 ml aliquots and store at –20 °C for max. 3 months.
Discard once thawed, not used up solution!

Reduction reagent: 1 % (w/v) Dithiothreitol (DTT, Cat. No. 20710)

Alkylating reagent: 5 % (= 260 mM) Iodoacetamide (Cat. No. 26710)

3.1.2. Protocol

Equilibration steps are carried out in a tightly closed reaction tube on a shaker with moderate speed.

1. Equilibration step
Incubate strip 10 – 15 min. in equilibration solution + 1 % (w/v) DTT at room temperature.

Decant the solution.

2. Equilibration step
Add equilibration solution + 5 % iodoacetamide (= 260 mM) (Cat. No. 26710) to the tube containing the IPG strip. Incubate for 10 – 15 min at room temperature.

Decant solution and proceed to SDS-PAGE.
Use the equilibrated IPG strip immediately for second dimension SDS-PAGE.

3.2. Performance of 2. dimension – vertical SDS-PAGE

3.2.1. Running buffer preparation

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. SDS-PAGE

1. Prepare 0.5 % agarose solution in 1x electrophoresis running buffer and cool down to 60 °C. Keep it warm until you are ready to use the agarose solution.

2. 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.

3. Rinse gel well with 1x electrophoresis running buffer, empty afterwards.

4. 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.

5. Immerse equilibrated IPG strips briefly in 1x electrophoresis running buffer.

6. Slide the strip into the gel. Align properly on the gel surface using a thin weighing spatula. The IPG strip must lie air-bubble free on the surface.

7. For fixing of the strip carefully pour 0.5 % agarose solution into the gel up to the edge of the cassette.

8. After solidifying of the agarose fill buffer chambers with running buffer.

9. Close the electrophoresis chamber and connect to power supply. Switch on power supply and begin electrophoresis.

   Conditions:

   At constant amperage (recommended): Start with 10 mA/gel for 10 min, adjust then limiting amperage for homogeneous gels to 20 mA/gel and for gradient gels to 25 mA/gel.

   Duration: 70 - 90 min (higher percentage and gradient gels run up to ca. 90 min)

At constant voltage: 150 V

Duration: ca. 90 min., (higher percentage and gradient gels have an accordingly longer running time)

10. On completion of electrophoresis, switch off power supply, disconnect the electrophoresis chamber, remove electrophoresis buffer and remove cassettes.

11. 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.

12. To remove the gel, carefully detach the plates so that the gel remains on one. Gels can now be stained or used for blotting.

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 *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 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 (Cat. No. 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 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.

4.2. Silver staining (Blum et al., modified)

(Electrophoresis 1987, 8, 93-99)

4.2.1. Reagents and solutions

Fixing solution	20 % (v/v) ethanol (Cat. No. 11094), 5 % (v/v) acetic acid
Wash solution	30 % (v/v) ethanol
Pre-treatment solution	0.02 % sodium thiosulfate solution ($Na_2S_2O_3$ x 5 H_2O, 200 mg/l)
Staining solution	0.2 % silver nitrate solution ($AgNO_3$, 2 g/l)
Developer	6 % sodium carbonate solution (Na_2CO_3, 60 g/l, Cat. No. 30181) + 100 µl Formaldehyd (37 %) to 200 ml solution
Stop solution	10 % (v/v) acetic acid
Preservation solution	3 % (w/v) glycerol (Cat. No. 23176)

4.2.2. Protocol

Safety Precautions: *The solutions are highly inflammable, toxic and dangerous for the environment. Please follow the instructions for handling hazardous substances. It is strongly advised to wear protective glasses, gloves and clothing during all steps of the staining procedure.*

For optimal staining results please follow these guidelines:

- While handling gels wear only rubber gloves that have been rinsed with deionized water.
- Use clean containers and designate them for silver staining purposes only.
- Make sure the size of the container permits free movement of the gel during shaking and complete immersion of the gel in solution while staining.
- Do not put pressure on the gel while handling or changing solutions and never touch the gel with bare hands or metal objects
- Use teflon coated stir bars and clean glass containers to prepare reagents..
- Make staining solution and developer shortly before use.

The following staining steps are carried out on a shaker (50 – 100 rpm) in 100 ml solution per gel.

Step	**Solution**	**Duration**
1. Fixing	20 % (v/v) ethanol, 5 % (v/v) acetic acid	≥ 40 min.
2. Wash	30 % (v/v) ethanol	2 x 20 min.
3. Pre-treatment	0.02 % sodium thiosulfate solution ($Na_2S_2O_3$ x 5 H_2O, 200mg/l)	exakt 2 min.
4. Wash	H_2O dest.	3 x 20 sec.
5. Staining	0.2 % silver nitrate solution	40 min.
6. Wash	H_2O dest.	2 x 20 sec.
7. Developing	6 % sodium carbonate solution + 100 µl/200 ml formaldehyde (37 %)	After sight, ca. 2 - 10 min.
8. Wash	H_2O dest.	1 x 20 sec.
9. Stop	10 % (v/v) acetic acid	10 min.
10. Preservation	3 % (w/v) glycerol in H_2O dest.	20 min.

5. 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.

5.1. Tank blotting

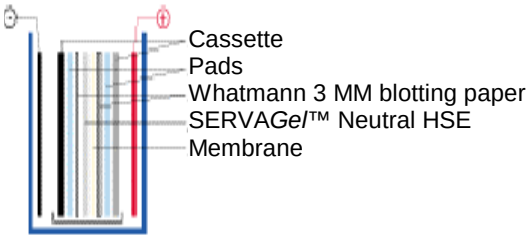
1. Cut transfer membrane and four pieces Whatmann 3 MM paper to gel size (7 x 8 cm).

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 (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).

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 (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.

6. Short protocol for 1. dimension with SERVA IPG*Blue* Strips

SERVA IPG *Blue*Strips are used in high resolution 2D-gel electrophoresis and guarantee a stable, reproducible gradient for IEF. For detailed instructions, refer to the SERVA IPG BlueStrips manual at www.serva.de or contact Technical Service.

6.1. Rehydration in rehydration tray

Rehydration-/Sample Buffer
Use concentration range in parenthesis as an optimization guideline for difficult samples.

Components	**Cat. No.**	**Concentration**	**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
Servalyt** pH 3-10 pH 4-7 pH 6-9 pH3-6	 42940 42948 42913 42944	 0.5 % (0.25 – 2 %) (absolutely)	125 µl
Water dest.			ad 10 ml

* replaceable up to 25 % by thiourea
**40 %, matching with pH-range of Strips

1. Immerse strip into **130 µl rehydration buffer** that way spreading liquid evenly (air-bubble free) over the whole length of the strip.

2. After absorption of solution (ca. 5-10 min.), overlay strip with 1 - 2 ml silicone oil and rehydrate for at least 6 hours (preferably over night) at room temperature.

6.2. Sample in-gel-rehydration:

Solve **5 - 100 µg total protein** in rehydration buffer and incubate the gel strip in it.

Cup Loading:
Rehydrate strip without sample, before IEF apply **5 - 100 µg total protein** to gel strip with the help of a silicone slot or the like (location of application depends on the gradient and the sample).

6.3. Focusing in an IEF flat bed chamber

- Place rehydrated gel strips **(gel side up)** in 1 –2 mm distance onto the silicone oil moistened cooling plate of the IEF flat bed chamber.

- Position a water dampened electrode wick on each gel end, add a little water or with water wetted cellulose sheets to the IEF chamber to prevent drying during focusing.

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

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 separation	Transfer gel to fixing or staining solution immediately after electrophoresis
	SDS quality in running buffer not suitable	Use higher quality SDS
Formation of stripes	Lipophilic substances in the sample	Remove substances prior to electrophoresis; increase SDS concentration if necessary

8. Order information

Precast gels	**Kat-Nr.**
SERVAGel™ TG PRiME™ 12 2D (10 Fertiggele)	43268.01
SERVAGel™ TG PRiME™ 12 2D (2 Fertiggele)	43268.03
SERVAGel™ TG PRiME™ 14 2D (10 Fertiggele)	43271.01
SERVAGel™ TG PRiME™ 14 2D (2 Fertiggele)	43271.03
SERVAGel™ Neutral HSE 2D (10 Fertiggele)	43247.01
SERVAGel™ Neutral HSE 2D (2 Fertiggele)	43247.03
SERVA IPG *Blue*Strip 3-10/7 cm	43001.01
SERVA IPG *Blue*Strip 3-10 NL/7 cm	43002.01
SERVA IPG *Blue*Strip 3-6/7 cm	43005.01
SERVA IPG *Blue*Strip 4-7/7 cm	43003.01
SERVA IPG *Blue*Strip 6-10/7 cm	43004.01
Equipment	
BlueVertical PRiME™ Mini Slab Gel System BV 102	BV 102
BlueFlash Semi-Dry Blotter Medium (15 x 15 cm)	BF-M
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
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
SERVA Proteome Markers	39220.01
Staining reagents and kits:	
SERVA *Densi*Stain Blue G Staining Solution (2x concentrate, 500 ml)	35078.01
SERVA Blue R Staining Kit (2 x 500 ml)	42531.01
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 black 10 B (50 g)	12310.01
Ponceau S solution (0,2 %, 500 ml)	33427.01
Silver nitrate	35110
Buffer 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 for SDS PAGE (10x)	42556
Towbin buffer 10x, for native PAGE and for Western Blotting	42558
Semi-Dry blotting buffer kit (3 x 500 ml)	42559

Glycine	23390
Tris(hydroxymethyl)aminomethane	37186
Brompophenol blue, sodium salt	15375
Dithiothreitol	20710
Iodoacetamide	26710
Ethanol, undenatured, absolute	11093
Glycerol	23176
2-Mercaptoethanol	28625
SDS in Pellets	20765
SDS solution, 20 % (w/v)	20767
Trichloroacetic acid, 20 % solution	36913
Silicone DC 200 fluid 10 cst	35132
Urea	24524
CHAPS	17038
Triton® X-100	39795
SERVALYT™ pH 3-10	42940
SERVALYT™ pH 4-7	42948
SERVALYT™ pH 6-9	42913
SERVALYT™ pH 3-6	42944
Membranes	
Immobilon (PVDF), 26.5 cm x 3.75 m, Porengröße: 0.2 µm (1 roll)	42574.01
Fluorobind (PVDF), 10 x 10 cm, Porengröße: 0.2 µm (20 sheets)	42573.01
Fluorobind (PVDF), 25 cm x 3 m, Porengröße: 0.2 µm (1 roll)	42571.01

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