

INSTRUCTION MANUAL

SERVA^{Ge}™ TG PRiME Starter Kit

Precast Vertical Gels for Electrophoresis

(Cat. No. 43206)



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1. SERVAGe™ TG PRiME™

1.1. General information

SERVAGe™ TG PRiME™ gels are ready-to-use Tris-Glycine gels, which are designed for vertical slab gel electrophoresis. They are suitable for fast (35 min) discontinuous separation according to Laemmli (Nature 277, 680 [1970]).

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™, 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. Kit components

SERVAGe™ TG PRiME™	4 gels
10 x Laemmli running buffer (Cat. No. 42556)	400 ml
2x Tris/Glycine-SDS sample buffer (Cat. No. 42527)	1 ml
Dithiothreitol (DTT, Cat. No. 20710)	310 mg (to be diluted in 1 ml H ₂ O)
SERVA Unstained SDS PAGE Protein Marker (Cat. No. 39215)	50 µl

Cassette:

Outer dimensions	10 cm x 10 cm
Number of sample wells	10 or 12
Volume of well	50 µl for the 10-well gels and 35 µl for the 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

SERVAGe™ TG PRiME™ gels are offered at various acrylamide concentrations (T). The gels contain **no SDS**. The separation ranges of gels for denatured proteins are shown in table 3.1.

Acrylamide concentration (T):	8 %, 10 %, 12 %, 14 % 4-12 %, 4-20 %, 8-16 %
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1.4. Storage conditions

Kit component	Storage Temperature
SERVAGe™ TG PRiME™	+2 °C – +8 °C
10 x Laemmli running buffer (Kat.-Nr. 42556)	+15 °C – +30 °C
2x Tris/Glycine-SDS sample buffer (Kat.-Nr. 42527)	+2 °C – +8 °C
Dithiothreitol (DTT, Kat.-Nr. 20710)	+2 °C – +8 °C
SERVA Unstained SDS PAGE Protein Marker (Kat.-Nr. 39215)	-15 °C – -30 °C

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 protocols

3.1. Separation range of gels

Acrylamide concentration (%)	Separation range (Mr 10 ³)
8	40 - 250
10	30 - 200
12	20 - 150
14	10 - 100
4 - 12	30 - 300
8 - 16	20 - 250
4 - 20	6 - 200

3.2. Running buffer preparation

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

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

3.3. Sample preparation

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.

Sample buffer components	Concentration 2x 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 samples for 5 minutes at 65 °C.
- Rinse wells with running buffer.
- Load samples and start electrophoresis.

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 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:

Please fill the electrophoresis chamber up to 90 % with running buffer.

Homogenous gels: 8 %, 10 %, 12 %, 14 %

Voltage	250 V	230 V
Current	50 mA / Gel	10 min: 10 mA / Gel ca. 50 min: 20 mA / Gel
Time	ca. 35 – 40 min	ca. 60 min

Gradient gels: 4-12 %, 4-20 %, 8-16 %

Settings Voltage	350 V	
Settings Current	50 mA / Gel	10 min: 10 mA / Gel ca. 90 min: 20 mA / Gel
Time	ca. 40 min	ca. 100 min

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 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 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 (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.

Native gels: Fix gel in 20 % (w/v) trichloroacetic acid for 30 min., wash gel for 1 min. in distilled water before staining.

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.

5. Protein transfer

Safety information:

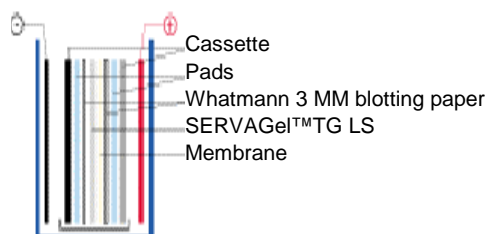
For safety reasons, always wear protective gloves and clothing, when working with gels and buffer solutions.

Blotting of SERVAGel™ TG PRiME™ 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 the membrane 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 and equilibrate the gel 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 bis 2 hours.

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 the membrane 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 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 0.8 - 1.5 mA/cm² gel area for ca. 1 hour.

By blotting of proteins of differently large sizes 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) and 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. 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
	SDS quality in running buffer not suitable	Use higher quality SDS
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)

7. Order information

Precast gels	Cat No.
SERVAGel™ TG PRiME™ 8, 12 sample wells	43260
SERVAGel™ TG PRiME™ 8, 10 sample wells	43261
SERVAGel™ TG PRiME™ 10, 12 sample wells	43263
SERVAGel™ TG PRiME™ 10, 10 sample wells	43264
SERVAGel™ TG PRiME™ 12, 12 sample wells	43266
SERVAGel™ TG PRiME™ 12, 10 sample wells	43267
SERVAGel™ TG PRiME™ 12, 2D sample well	43268
SERVAGel™ TG PRiME™ 14, 12 sample wells	43269
SERVAGel™ TG PRiME™ 14, 10 sample wells	43270
SERVAGel™ TG PRiME™ 14, 2D sample well	43271
SERVAGel™ TG PRiME™ 4-12, 12 sample wells	43273
SERVAGel™ TG PRiME™ 4-12, 10 sample wells	43274
SERVAGel™ TG PRiME™ 4-20, 12 sample wells	43276
SERVAGel™ TG PRiME™ 4-20, 10 sample wells	43277
SERVAGel™ TG PRiME™ 8-16, 12 sample wells	43279
SERVAGel™ TG PRiME™ 8-16, 10 sample wells	43280
SERVAGel™ TG PRiME™ 8-16, 2D well	43281
SERVAGel™ Neutral HSE, 12 sample wells	43245
SERVAGel™ Neutral HSE, 10 sample wells	43246
SERVAGel™ Neutral HSE, 2D	43247
SERVAGel™ Neutral pH 7.4, 12 sample wells	43220
SERVAGel™ Neutral pH 7.4, 10 sample wells	43222
SERVAGel™ Neutral pH 7.4 Gradient, 12 sample wells	43221
SERVAGel™ Neutral pH 7.4 Gradient, 10 sample wells	43223
SERVAGel™ Neutral HSE Starter Kit	43207
Equipment	
BlueVertical PRiME Mini slab gel system	BV 104
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 <i>DensiStain</i> Blue G Staining Solution (2x concentrated, 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 black10 B (50 g)	12310.01
Staining reagents and kits:	
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
Bromophenol blue, sodium salt	15375
Dithiothreitol	20710
Ethanol, undenatured, absolute	11093
Glycerol	23176
2-Mercaptoethanol	28625
SDS in Pellets	20765
SDS solution, 20 % (w/v)	20767
Trichloroacetic acid, 20 % solution	36913
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), 10 x 10 cm, pore size: 0.2 µm (20 sheets)	42573.01
Fluorobind (PVDF), 25 cm x 3 m, pore size: 0.2 µm (1 roll)	42571.01

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