INSTRUCTION MANUAL

SERVA*Gel*™ SDS PAGE Starter Kit

Precast Vertical Gels for Electrophoresis

(Cat. No. 43200.01)

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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1. SERVA*Gel*™ SDS PAGE Starter Kit

1.1. General information

SERVA*Gel*™ SDS PAGE Starter Kit contains in addition to ready-to-use Tris/Glycine gels all reagents you need for performing SDS PAGE. The included SERVA*Gel*™ TG gels are designed for vertical slab gel electrophoresis and suited for discontinuous separation according to Laemmli (Nature 277, 680 [1970]). They do not contain SDS and thus, can also be operated with other (e.g., native) buffers. SERVA offers homogenous or gradient precast gels featuring various acrylamide concentrations (T).

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. Kit components

SERVA*Gel*™ Tris/Glycine gels (Cat. No. selectable)	4 pieces
Tool for opening of cassette	1 piece
10x 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 (for 1ml H_2O)
SERVA unstained SDS PAGE protein marker (Cat. No. 39215)	50 µl

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.

Cassette :

Outer dimensions	10 cm x 10 cm
Number of sample wells	12
Volume of well	35 µl

Gel:

Material	Acrylamide/N, N'-methylene bisacrylamide
Dimensions separation gel	Length 7 cm x width 8 cm
Thickness of gel layer	1 mm

Note:
Reagents needed for the subsequently staining of the gels are not included in the kit and must be ordered separately. A detailed Western Blotting protocol for the SERVA*Gel*™ TG gels is available on request:
Phone: +49-6221-1384044
E-mail: tech.service@serva.de

1.3. Composition of gels

SERVA*Gel*™ TG gels are offered at various acrylamide concentrations (T). The gels contain **no SDS**. Depending on the electrophoresis buffer used, you determine whether native or denaturing conditions prevail. The separation ranges of gels for denatured proteins are shown in table 3.1.(p. 22)

Acrylamide concentration (T):	8 %, 10 %, 12 %, 14 %, 16 %, 4 – 12 %, 8 – 16 %, 4 – 20 %
Cross linker concentration (C):	2.6 %
Stacking gel:	4 % T, 2.6 % C
Gel buffer:	
Stacking gel	125 mM Tris/HCl, pH 6.8
Separation gel	375 mM Tris/HCl, pH 8.8

1.4. Storage conditions

Kit components	Storage temperature
10x Laemmli running buffer	+15 ℃ - +30 ℃
SERVA*Gel*™ TG gels	2 – 8 ℃
2x Tris/Glycine/SDS sample buffer	2 – 8 ℃
Dithiothreitol (DTT)	2 – 8 ℃
SERVA unstained SDS PAGE protein marker	-15 ℃ – 25 ℃

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.

Please store SERVA unstained SDS PAGE protein marker 6.5 – 200 kDa, liquid mix at –20 ℃. The marker may be stored (a few days) at 4 ℃ for a short time. To prevent frequent freeze-thaw cycles the protein marker should be frozen in aliquots.

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 ℃. 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.

Reagents needed for the subsequently staining of the gels are not included in the kit and must be ordered separately. A detailed Western Blotting protocol for the SERVA*Gel*™ TG gels is available on request:
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3. Electrophoresis protocols

3.1. Separation range of gels

Acrylamide concentration (%)	Separation range (Mr 10^3)
8	40 - 250
10	30 - 200
12	20 - 200
14	10 – 100
16	5 - 70
4 - 12	30 - 300
8 - 16	20 - 250
4 - 20	6 - 200

3.2. Running buffer preparation

3.2.1. Denaturing conditions (SDS)

Dilute **10x Laemmli running buffer** 1:10 (composition see appendix, page 27), pH value 8.8.

3.3. Sample preparation

The **2x Tris-Glycine/SDS sample buffer** (composition see appendix, page 27) does not contain any reduction reagent. By adding 10 mM DTT 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.

- **Preparation of 2 M DTT stock solution:**
  Solve the content of the DTT vial (310 mg) in 1 ml water, deion. The stock solution should be stored at –20 ℃.
- Add **10 µl of the 2 M DTT stock solution** to 1 ml 2x Tris/Glycine-SDS sample buffer.
- Samples are diluted (1:1) with an equal volume of 2x sample buffer and mixed well. The maximal well volume is 35 µl.
- Heat sample for 5 minutes at 95 ℃; heat fluorescen ce-labelled samples for 5 minutes at 65 ℃.
- Rinse wells with running buffer.
- Load samples and start electrophoresis.

3.3.1. 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

3.3.2. Instructions for use SERVA Unstained SDS PAGE Protein Marker

SERVA unstained SDS PAGE protein marker is a ready-to-use protein marker for SDS PAGE. It contains 8 standard proteins of 6.5 kDa to 200 kDa in Tris/Glycine-SDS sample buffer (with 10 mM DTT).

- Warm up protein marker solution to room temperature to dissolve again precipitated SDS.
  Important: Do not boil marker, just warm up in water bath for a short time.

  The protein marker is pre-reduced and acylated. Eventually occurring aggregates (at barrier to separation gel) can be dissolved by warming up to 80℃ /do not exceed temperature limit!) for 2 - 5 min.

- For **staining** of gel with **Coomassie™, SERVA Blue G or SERVA Blue R** appy **5 µl** of protein standard per well.

- For **silver staining dilute** protein marker **1:5** in 1x Tris/Glycin-SDS PAGE sample buffer and then apply **5 µl** per well.

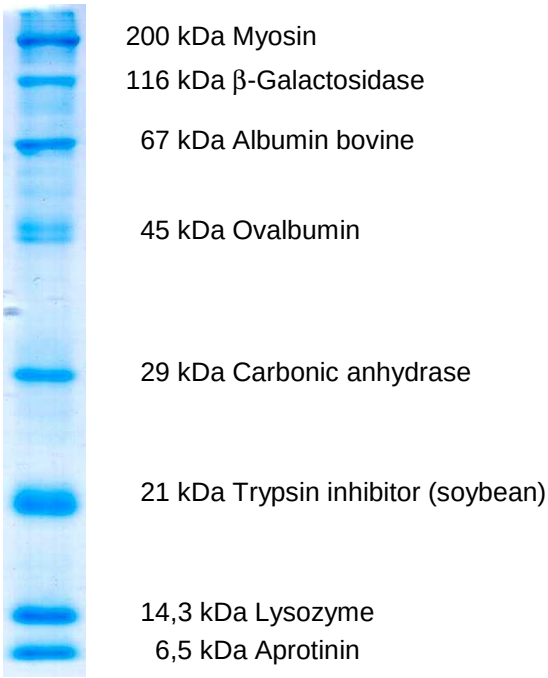

Figure 1: Separation of 5 µl of **SERVA Unstained SDS PAGE Protein Marker** on 12 % SDS-PAA-gel.

3.4. Electrophoresis conditions

Electrophoresis is carried out under the following conditions:

Let samples run into the gel for 15 minutes at 10 mA/gel.
Adjust then limiting amperage for homogeneous gels to 20 mA/gel and for gradient gels to 25 mA/gel.
Voltage will rise during the run from initial ca. 60 V to 250 V.
Duration: 70 - 90 min (higher percentage and gradient gels run up to ca. 90 min.)
We recommend running the gel at constant amperage.

Alternatively gels can be run at constant voltage of 150 V. Amperage falls during run from initial 20 – 25 mA to ca. 10 mA. **Duration:** ca. 90 min., (higher percentage and gradient gels have an accordingly longer running time)

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 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.

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. 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)

5. Appendix

Composition of buffers:

10x Laemmli running buffer

Components	Concentration
Tris	0.25 M
Glycine	1.92 M
SDS	1 %

2x Tris/Glycine-SDS sample buffer

Components	Concentration
1 M Tris-HCl pH 6.8	0.126 M
10 % (w/v) SDS	4 %
Glycerol	20 %
0.1 % (w/v) Bromophenol blue	0.02 %
2 M DTT	0.02 M
Or: 2-Mercaptoethanol	*10 %*
Water, deion.	

7. Order information

Precast gels	**Cat No.**
SERVAGel™ TG 8 %Tris-Glycine (10 gels)	43208.01
SERVAGel™ TG 8 %Tris-Glycine (6 gels)	43208.02
SERVAGel™ TG 8 %Tris-Glycine (2 gels)	43208.03
SERVAGel™ TG 10 % Tris-Glycine (10 gels)	43210.01
SERVAGel™ TG 10 % Tris-Glycine (6 gels)	43210.02
SERVAGel™ TG 10 % Tris-Glycine (2 gels)	43210.03
SERVAGel™ TG 12 % Tris-Glycine (10 gels)	43212.01
SERVAGel™ TG 12 % Tris-Glycine (6 gels)	43212.02
SERVAGel™ TG 12 % Tris-Glycine (2 gels)	43212.03
SERVAGel™ TG 14 % Tris-Glycine (10 gels)	43214.01
SERVAGel™ TG 14 % Tris-Glycine (6 gels)	43214.02
SERVAGel™ TG 14 % Tris-Glycine (2 gels)	43214.03
SERVAGel™ TG 16 % Tris-Glycine (10 gels)	43216.01
SERVAGel™ TG 16 % Tris-Glycine (6 gels)	43216.02
SERVAGel™ TG 16 % Tris-Glycine (2 gels)	43216.03
SERVAGel™ TG 4 - 20 % Tris-Glycine (10 Fertiggele)	43230.01
SERVAGel™ TG 4 - 20 % Tris-Glycine (6 Fertiggele)	43230.02
SERVAGel™ TG 4 - 20 % Tris-Glycine (2 Fertiggele)	43230.03
SERVAGel™ TG 8 - 16 % Tris-Glycine (10 Fertiggele)	43231.01
SERVAGel™ TG 8 - 16 % Tris-Glycine (6 Fertiggele)	43231.02
SERVAGel™ TG 8 - 16 % Tris-Glycine (2 Fertiggele)	43231.03
SERVAGel™ TG 4 - 12 % Tris-Glycine (10 Fertiggele)	43232.01
Precast gels	**Cat No.**
SERVAGel™ TG 4 - 12 % Tris-Glycine (6 Fertiggele)	43232.02
SERVAGel™ TG 4 - 12 % Tris-Glycine (2 Fertiggele)	43232.03
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 *Densi*Stain 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
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
Membranes	
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