INSTRUCTION MANUAL

SERVA*Gel*™N Native Gel Starter Kit

Precast Vertical Gels for Native Electrophoresis

(Cat. No. 43204.01)

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Vers. 02/10

1. SERVA*Gel*™N Native Gel Starter Kit

1.1. Introduction

1.1.1. Native gel electrophoresis

The analysis of protein mixtures is routinely performed using SDS-PAGE. Because of the denaturing method, the analysis of multi protein complexes is not possible.

Protein separation and analysis using native/non-denaturing conditions can be performed by using different methods, e.g. *Blue Native* or *Clear Native* electrophoresis.

1.1.2. *Blue Native* gel electrophoresis

Peforming *Blue Native* gel electrophoresis, the detergent SDS is substituted by Coomassie Brilliant Blue G250/SERVA Blue G. This dye is also negatively charged. Dye-binding to proteins leads to negatively charged protein dye complexes. The native structure of the proteins is preserved because the dye does not act as a detergent. At physiological pH, the protein dye complexes migrate pI-independently towards the anode. The repulsion between the negatively charged protein dye complexes leads to a high selectivity.

1.1.3. *Clear Native* gel electrophoresis

The *Clear Native* gel electrophoresis works without using an anionic dye. Therefore, this method can be used for separation of proteins with pI<7 at physiological pH when dyes may interfere with further analytical methods.

1.2. General Information

The SERVA*Gel*™N Native Gel Starter Kit contains ready-to-use native vertical gels, electrophoresis cathode and anode buffer as well as two different sample buffers to perform *Blue Native* (BN) and *Clear Native* (CN) gel 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, leakage-free plastic cassettes
- 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. Hoefer Mighty Small™ SE 260, Hoefer miniVE™, etc.)

The precast gels are manufactured according to proprietary methods developed by SERVA Electrophoresis GmbH and 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.

1.3. Kit components

SERVA*Gel*™N 3-12, Vertical Native Gel 3-12%	2 pieces
SERVA*Gel*™N 4-16, Vertical Native Gel 4-16%	2 pieces
Tool for gel cassette opening	1 piece
10x Native Anode Buffer for BN/CN	250 ml
10x Native Cathode Buffer for BN/CN	250 ml
SERVA Native Marker Liquid Mix for BN/CN	50 µl
2x Sample Buffer Blue Native	2 ml
2x Sample Buffer Clear Native	2 ml
1% SERVA Blue G solution for BN	5 ml

Gel cassette:

Outer dimensions	10 cm x 10 cm
Number of sample wells	10
Volume per well	50 µl

Gel:

Material	Acrylamide/N,N'-Methylenbisacrylamide
Thickness of gel layer	1 mm

Please note:

Reagents for gel staining are not included. These products have to be ordered separately.

1.4. Composition of gels

Acrylamide concentration (T): 3-12 %/4-16 %

Cross linker concentration (C): 2.6 %

1.5. Storage conditions

Kit component	**Storage temperature**
10x Native Anode and Cathode Buffer for BN/CN	+15 °C - +30 °C
SERVAGel™N Native Gele	2 – 8 °C
2x Sample Buffer Blue Native	-25 °C - -15°C
2x Sample Buffer Clear Native	-25 °C - -15°C
SERVA Native Marker Liquid Mix for BN/CN	-25 °C - -15°C
1% SERVA Blue G solution for BN	+15 °C - +30 °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 useable 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 protocols

3.1. *Blue Native* Electrophoresis (BN)

3.1.1. Running buffer preparation for BN electrophoresis

Dilute **10x Anode Buffer** 1:10 (composition see Appendix, page 28).

Dilute **10x Cathode Buffer** 1:10 (composition see Appendix, page 28) and add **1 % SERVA Blue G solution** to get a final concentration of 0.002 % (w/v), e.g. 1 ml Blue G solution in 500 ml 1x Cathode Buffer.

3.1.2. Sample preparation

- Mix your sample with the same volume **2x Sample Buffer for BN** (composition see Appendix, page 28). The maximum volume per well is 50 µl. **Do not heat samples!**
- Rinse wells with 1x Cathode Buffer.
- Load samples and start electrophoresis.

3.2. *Clear Native* Electrophoresis (CN)

3.2.1. Running buffer preparation for CN electrophoresis

Dilute **10x Anode Buffer** 1:10 (composition see Appendix, page 28)

Dilute **10x Cathode Buffer** 1:10 (composition see Appendix, page 28)

3.2.2. Sample preparation

- Mix your sample with the same volume 2x sample buffer for CN (composition see Appendix, page 28). The maximum volume per well is 50 µl. **Do not heat samples!**
- Rinse wells with 1x Cathode Buffer.
- Load samples and start electrophoresis.

3.3. Conditions for BN- and CN electrophoresis

Electrophoresis is carried out under the following conditions:

Voltage:

10 min	U = 50 V = const.
ca. 120 min	U = 200 V = const.

Amperage will decrease during run from initial ca. 15 mA/gel (200 V) to ca. 5 mA.

Duration: Due to running conditions varying from saple to sample, no standard protocol is available. The running conditions have to be defined by the user.

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 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

Fixation	20 % (w/v) trichloroacetic acid (Cat. No. 36913)
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	Fix gel in 20 % (w/v) trichloroacetic acid for 30 min., wash gel for 1 min. in distilled water before staining.
Staining	**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. Appendix

Composition of buffers:

10x Cathode Buffer for BN/CN: 500 mM Tricine (Serva Cat. No. 37195)
150 mM BisTris (Serva Cat. No. 15107)

10x Anode Buffer for BN/CN: 500 mM BisTris – HCl pH 7.0

2x Sample Buffer Blue Native:

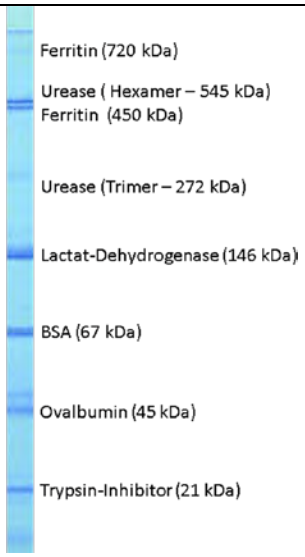
1 M 6-Aminocaproic acid (Serva Cat. No. 12548)
100 mM BisTris-HCl pH 7.0
100 mM NaCl (Serva Cat. No. 30183)
20 % Glycerol (Serva Cat. No. 23176)
0.1 % Serva Blue G250 (Serva Cat. No. 35050)
(+Detergent, sample-dependent)

2x Sample Buffer Clear Native:

100 mM NaCl (Serva Cat. No. 30183)
100 mM Imidazole (Serva Cat. No. 26081)
4 mM 6-Aminocaproic acid (Serva Cat. No. 12548)
2 mM EDTA
0.02 % Ponceau S (Serva Cat. No. 33429)
20 % Glycerol (Serva Cat. No. 23395)

SERVA Native Marker Liquid Mix for BN/CN Cat. No. 39219

Product Description:

General	Ready-to-use protein standard for Blue Native (BN) and Clear Native (CN) Electrophoresis containing 6 different proteins with a molecular weight range between 21 and 720 kDa. .
Application	• Blue Native and Clear Native electrophoresis
	
Storage	-20 °C, Storage in small aliquots is recommended to avoid repeated freeze/thaw cycles.
Operating instruction	Recommended loading volume: • 5 – 10 µl/lane (gel size 10 x 8 cm, 0.75 oder 1 mm thickness) • 10 – 15 µl/lane (gel size 30 x 20 cm, 1 oder 1.5 mm thickness)

6. Order Information

Product	Cat. No.
Precast gels	
SERVA*Gel*™ N 3-12, Vertical Native Gel 3-12 % 12 wells	43250
SERVA*Gel*™ N 3-12, Vertical Native Gel 3-12 % 10 wells	43251
SERVA*Gel*™N 4-16, Vertical Native Gel 4-16 % 12 wells	43253
SERVA*Gel*™N 4-16, Vertical Native Gel 4-16 % 10 wells	43252
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*™ Neutral HSE, 12 sample wells	43245
SERVA*Gel*™ Neutral HSE, 10 sample wells	43246
SERVA*Gel*™ Neutral HSE, 2D	43247
SERVA*Gel*™ Neutral pH7.4, 12 sample wells	43220
SERVA*Gel*™ Neutral pH7.4, 10 sample wells	43222
SERVA*Gel*™ Neutral pH7.4 Gradient, 12 sample wells	43221
SERVA*Gel*™ Neutral pH7.4 Gradient, 10 sample wells	43223
SERVA*Gel*™ Neutral HSE Starter Kit	43207
SERVA*Gel*™ PRiME™ Starter Kit	43206
Equipment	
BlueVertical PRiME™ Mini Slab Gel System BV 102	BV 102
Blue Power 500x4 Power Supply	BP-500x4
BlueFlash Semi-Dry Blotter Medium (15 x 15 cm)	BF-M
Protein Standards	
SERVA Native Marker Liquid Mix for BN/CN	39219.01
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 Native PAGE (25 Minigele)	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

Buffers and Solutions	
10x Native Anode Buffer for BN/CN	42535.01
10x Native Cathode Buffer for BN/CN	42536.01
2x Sample Buffer Blue Native	42533.01
2x Sample Buffer Clear Native	42534.01
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
Ethanol, undenatured, absolute	11093
Glycerol	23176
Trichloroacetic acid, 20 % solution	36913
SERVA Blue G Solution for BN, 1 %	42538.01
Membranes	
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