INSTRUCTION MANUAL

SERVAGel™ IEF 3-10

Precast Vertical Gels for Isoelectric Focusing

(Cat. No. 43240.01, 43242.01)

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1. SERVA*Gel*™ IEF 3-10

1.1. General Information

The SERVA*Gel*™ IEF 3-10 are ready-to-use gels for vertical isoelectric focusing. The gels are suitable for standard IEF with cathodic sample application as well as for NEPHGE (non-equilibrium pH gradient electrophoresis) with sample application at the anode. This allows optimal protein analysis in the acidic as well as the basic pH range of the gel including pH 8.5 to 10.7.

These gels are also included in the SERVA*Gel*™ IEF Starter Kit (Cat. No. 43205.01). This kit contains also electrophoresis cathode and anode buffer as well as sample buffer.

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.

Gel cassette:

Outer dimensions	10 cm x 10 cm
Number of sample wells	10 / 12
Volume per well	50 µl / 35 µl

Gel:

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

1.2. Storage conditions

Store the gels at 2 – 8 °C upon arrival.
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 °C - +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 section 3 and 4.

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 IEF protocol

3.1. Running buffer preparation for IEF

3.1.1. Cathode Buffer

Solubilize **SERVA IEF cathode buffer** in **1 l** bidist. water. 200 ml buffer is normally sufficient for filling the inner cathode chamber of the electrophoresis unit.

3.1.2. Anode Buffer

Prepare the anode buffer **not less then one hour before starting the IEF**, this will make sure that the anode buffer powder will be solved properly.

Solubilize **SERVA IEF anode buffer** in **2.5 l** bidist. water by stirring at room temperature. For complete filling of the outer buffer chamber (anode) ca. 500 ml buffer (depending on chamber size) should be sufficient.

Note: Do not use any other anodic buffer as described. The use of phosphoric acid as anode buffer will cause severe disturbances during IEF.

3.2. Sample preparation for IEF

- Mix your sample with the same volume **IEF sample buffer**. The maximum volume per well is 50 µl (10 sample wells) and 35 µl (12 sample wells).
  Do not heat samples!
- Rinse wells with cathode buffer.
- Load samples and start electrophoresis.

3.3. Conditions for IEF

Electrophoresis is carried out under the following conditions:

Voltage:

60 min	U = 50 V = const.
60 min	U = 200 V = const.
30 min	U = 500 V = const.

Amperage will decrease during run from initial ca. 8 mA/gel (100 V) to ca. 6 mA.

4. NEPHGE protocol

Important: For NEPHGE the polarity of the electrophoresis unit (compared to standard IEF) has to be changed.
Please note that gels are cooled during the electrophoresis (the running buffer temperature should not exceed 20 °C).

4.1. Running buffer preparation

Anode buffer (1x): 40 mM glutamic acid

Cathode buffer (1x): 20 mM NaOH

4.2. Preparations for NEPHGE

- Mix your sample with the same volume **IEF sample buffer**. The maximum volume per well is 50 µl (10 sample wells) and 35 µl (12 sample wells).
  Do not heat samples!
- Fill the inner buffer chamber with anode buffer and rinse wells of the gels with anode buffer.
- Fill the complete outer buffer chamber with cathode buffer.
- Load the samples.
- Connect the inner buffer chamber with the anode (+) and the outer buffer chamber with the cathode (-).
- Start the electrophoresis.

4.3. Conditions for NEPHGE

NEPHGE is carried out using the following conditions:

Voltage:

60 min	U = 100 V = const.
20 min	U = 200 V = const.
5 min*	U = 500 V = const.

* Depending on the samples, this step can be extended up to 10 min. Then Cytochrome C (pI = 10.7) will not be detectable in the gel anymore.

5. Staining with SERVA Violet 17

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

5.1. Reagents and solutions

Fixation	20 % (w/v) trichloroacetic acid solution (Cat. No. 36913)
Stock solution 1	0.2 % SERVA Violet 17 (Cat No. 35072) in bidist. water (100 mg SERVA Violet 17 in 50 ml water)
Stock solution 2	20 % (w/v) phosphoric acid (140 ml 85 % H_3PO_4 in 1000 ml bidist. water)
Destainer	3 % (w/v) phosphoric acid (w/v) (20 ml 85 % H_3PO_4 in 1000 ml bidist. water)
Preservation solution	30 % (v/v) ethanol, 5 % (w/v) glycerol

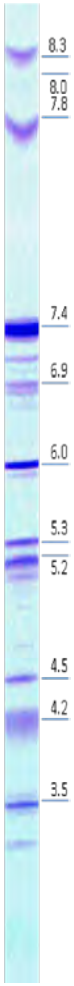
5.2. Protocol

Carry out all fixing and staining steps 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 10 min. in the solution.
Destainer	Rinse gel after staining for **1 minute with dist. water** and incubate in destainer until the background is clear.
Preservation	Incubate gel over night in preservation solution. The gel can then be dried in a drying frame.

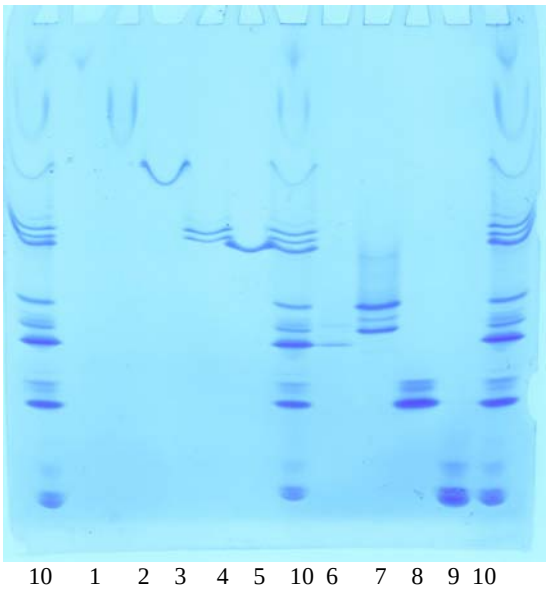
6. Appendix

SERVA IEF Marker 3-10 Liquid Mix (Cat. No. 39212.01)



Protein	pI
Lectin (Lens culinaris)	8.3, 8.0, 7.8
Myoglobin	7.4, 6.9
Carbonic anhydrase	6.0
ß-Lactoglobulin	5.3, 5.2
Trypsin inhibitor	4.5
Glucose oxidase	4.2
Amyloglucosidase	3.5

NEPHGE using different marker proteins and SERVA IEF Marker 3-10 Liquid Mix (Cat. No. 39212.01)



1: Amyloglucosidase pI 3,5
2: Glucoseoxidase pI 4,2
3: Trypsininhibitor pI 4,5
4: ß-Lactoglobulin pI 5,3 und 5,2
5: Carbonanhydrase pI 6,0
6: Myogobin pI 6,9 und 7,4
7: Lectin pI 7,8/8,0/8,3
8: Ribonuclease A pI 9,5
9: Cytochrom C pI 10,7
10:SERVA IEF Marker 3-10 Liquid Mix

6. Order Information

Product	Cat. No.
Precast Gels	
SERVA*Gel*™ IEF 3-10, 12 wells	43240.01
SERVA*Gel*™ IEF 3-10, 10 wells	43242.01
SERVA*Gel*™ N 3-12, Vertical Native Gel 3-12 % 12 wells	43250.01
SERVA*Gel*™ N 3-12, Vertical Native Gel 3-12 % 10 wells	43251.01
SERVA*Gel*™N 4-16, Vertical Native Gel 4-16 % 12 wells	43252.01
SERVA*Gel*™N 4-16, Vertical Native Gel 4-16 % 10 wells	43253.01
SERVA*Gel*™ Neutral pH7.4, 12 sample wells	43220.01
SERVA*Gel*™ Neutral pH7.4, 10 sample wells	43222.01
SERVA*Gel*™ Neutral pH7.4 Gradient, 12 sample wells	43221.01
SERVA*Gel*™ Neutral pH7.4 Gradient, 12 sample wells	43223.01
SERVA*Gel*™ PRiME™ 8, 12 sample wells	43260.01
SERVA*Gel*™ PRiME™ 8, 10 sample wells	43261.01
SERVA*Gel*™ PRiME™ 10, 12 sample wells	43263.01
SERVA*Gel*™ PRiME™ 10, 10 sample wells	43264.01
SERVA*Gel*™ PRiME™ 12, 12 sample wells	43266.01
SERVA*Gel*™ PRiME™ 12, 10 sample wells	43267.01
SERVA*Gel*™ PRiME™ 12, 2D sample well	43268.01
SERVA*Gel*™ PRiME™ 14, 12 sample wells	43269.01
SERVA*Gel*™ PRiME™ 14, 10 sample wells	43270.01
SERVA*Gel*™ PRiME™ 14, 2D sample well	43271.01
SERVA*Gel*™ PRiME™ 4-12, 12 sample wells	43273.01
SERVA*Gel*™ PRiME™ 4-12, 10 sample wells	43274.01
SERVA*Gel*™ PRiME™ 4-20, 12 sample wells	43276.01
SERVA*Gel*™ PRiME™ 4-20, 10 sample wells	43277.01
SERVA*Gel*™ PRiME™ 8-16, 12 sample wells	43279.01
SERVA*Gel*™ PRiME™ 8-16, 10 sample wells	43280.01
SERVA*Gel*™ PRiME™ Starter Kit	43206.01
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
SERVA IEF Marker 3-10 Liquid Mix	39212.01
Protein Test Mixture for pI-Determination pH 3-10, lyophil.	39211.01

Product	Cat. No.
Staining Reagents and Kits:	
SERVA *Densi*Stain Blue G Staining Solution (2-fold conc., 500 ml)	35078.01
SERVA Blue R Staining Kit (2 x 500 ml)	42531.01
SERVA Silver Staining Kit Native PAGE (25 mini gels)	35077.01
SERVA Blue G	35050.01
SERVA Blue R	35051.01
Amido black 10 B (50 g)	12310.01
Ponceau S solution (0.2 %, 500 ml)	33427.01
Silver nitrate	35110.01
Buffers and Solutions	
SERVA*Gel*™ IEF Running Buffer Kit	42539.01
IEF Sample Buffer (2x)	42537.01
Native Anode Buffer for BN/CN (10x)	42535.01
Native Cathode Buffer for BN/CN (10x)	42536.01
Sample Buffer Blue Native (2x)	42533.01
Sample Buffer Clear Native (2x)	42534.01
SERVA Tris-Glycine native electrophoresis buffer (10x)	42530.01
SERVA Tris-Glycine native sample buffer (2x)	42528.01
Laemmli Buffer 10x, for SDS PAGE	42556.01
Laemmli Sample Buffer 2x, for SDS PAGE	42526.01
SERVA Tris-MOPS/SDS electrophoresis buffer (20x)	42561.01
SERVA Tris-Tricine/SDS electrophoresis buffer (20x)	42560.01
SERVA Tris-Tricine/SDS sample buffer (2x)	42551.01
Towbin buffer 10x, for native PAGE and for Western Blotting	42558.01
Semi-Dry Blotting buffer kit (3 x 500 ml)	42559.01
Glycine	23390.01
Tris(hydroxymethyl)aminomethane	37186.01
Brompophenol blue, sodium salt	15375.01
Ethanol, undenatured, absolute	11093.01
Glycerol	23176.01
Trichloroacetic acid, 20 % solution	36913.01
SERVA Blue G Solution for BN, 1 %	42538.01
Membranes	
Immobilon™-P-membrane (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

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