

Product Guide

VELUM Precast 1D Gels

Product no. PR223, PR227, PR254, PR255, PR256, PR257

NH DyeAGNOSTICS GmbH
Weinbergweg 23
D-06120 Halle

Technical Support
Fon: +49 (0) 345-2799 6413
e-mail: service@dyeagnostics.com
www.dyeagnostics.com

copyright © NH DyeAGNOSTICS © 2017
Stand 10/2017 (1)

1 Kit content

- 4 VELUM Precast 1D Gels (12.5% AA/BisAA; size: 250 x 85 x 0.5 mm)
- VELUM Precast 1D Gel Cathode Strip (colorless)
- VELUM Precast 1D Gel Anode Strip (blue)
- VELUM Precast 1D Gel Sample Buffer
- VELUM Precast Gel Cooling Solution

2 Additional material required (not included)

- Dithiothreitol (DTT)
- ORCA Gel Electrophoresis System or horizontal electrophoresis system

3 Storage and stability

See package label

4 Gel types

Product no.	Gel	Slots	Slot volume	suitable for fluorescence detection
PR 254	VELUM GOLD Precast 1D Gels	25	12 µl	blue, green, red and infrared
PR 255	VELUM GOLD Precast 1D Gels	52	5 µl	blue, green, red and infrared
PR 256	VELUM SILVER Precast 1D Gels	25	12 µl	red and infrared
PR 257	VELUM SILVER Precast 1D Gels	52	5 µl	red and infrared

5 General information

VELUM Precast Gels are designed for high resolution horizontal protein separation of complex protein samples. Due to a film-backing support VELUM Precast Gels do not require glass plates and run with very small amounts of buffer (included within the kit).

VELUM GOLD Precast 1D Gels are low fluorescent and therefore ideal for fluorescent labeling and staining in a spectrum from blue to infrared and also for common Coomassie staining.

VELUM SILVER Precast 1D Gels are ideal for fluorescent labeling and staining in a red to infrared spectra as well as common Coomassie- und SILVER staining.

6 Instructions for use

6.1 Preparation of the horizontal electrophoresis system

- Clean the cooling plate and the electrodes with ddH₂O using a lintfree tissue.
- Apply 2 ml VELUM Precast Gel Cooling Solution onto the cooling plate along the center line.

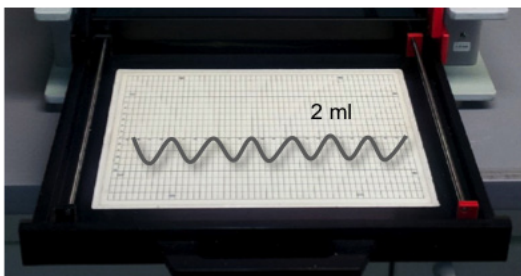


Fig. 1 Application of Cooling Solution

6.2 Placing the Gel

- Replace the upper film support from the VELUM Precast Gel (keep the upper film support and the outer packaging for the subsequent storage of the gel)
- Place the gel with the film support downwards and the wells towards you onto the VELUM Precast Gel Cooling Solution.

Caution: Avoid air bubbles between gel and cooling plate.

- Remove remaining VELUM Precast Gel Cooling Solution using lintfree tissues.

6.3 Placing the electrode Strips

- Place the drained Anode Strip (blue) onto the anodal edge (far from wells). Strip-gel-overlap: 3mm
- Place the drained Cathode Strip (colorless) onto the cathodal edge (sample side). Strip-gel-overlap: 3mm

Caution: Ensure proper gel-strip-contact by carefully pressing the strip on the gel

6.4 Sample preparation and sample application

6.4.1 Preparation of the loading buffer

For preparation of loading buffer mix

900 μ l VELUM Precast 1D Gels Sample Buffer
+ 100 μ l 1% DTT solution (1% (w/v) DTT in ddH₂O).

Note: Use newly prepared DTT solution.

6.4.2 Preparation and application of the samples

- Mix sample and newly prepared loading buffer (incl. DTT; see 6.4.1) in the ratio 1:2 (at least).
- incubate at 95 °C for 5 min.

- Apply the prepared samples into the gel slots. The maximum load capacity is 12 μ l for 25 slot and 5 μ l for 52 slot VELUM Precast 1D Gel, respectively.
- Load VELUM Precast 1D Gel Sample Buffer into empty wells. Do not leave wells unfilled.
- for a small number of samples halve the gel.

Note: If you are using one half of a gel only, cut the precast gel and the electrode strips into half. Adapt electrophoretic settings by reducing the current (mA) and power (W) by 50%. Use the voltage (V) and time settings as indicated in the table (page 3).

6.5 Placing the electrodes

ORCA Gel Electrophoresis Unit:

Place each of the cleaned platinum electrodes onto the electrode strips (gel far side). Apply negative voltage (-) onto the cathodal strip (colorless, sample side) and the positive voltage (+) onto the anodal strip (blue, sample far side). Press the platinum electrodes onto the strips to ensure optimal contact.

Other horizontal electrophoresis system:

Place the electrodes regarding to manufacturers' instructions onto the outside edge of the electrode strips and apply positive and negative voltage regarding to manufacturers declaration (cathodal strip at sample side, anodal strip at sample far side).

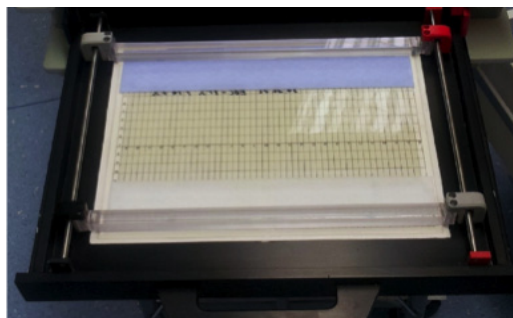


Fig. 2 Arrangement of gel, strips and electrodes

6.6 Gel electrophoresis

- Start the gel electrophoresis.
- use the following parameters for the run of one gel:

VELUM GOLD/SILVER Precast 1D Gels (PR254, PR255, PR256, PR257): ca. 2,5 h		
Parameter	Step 1	Step 2
Voltage	250 V	550 V
Current	20 mA	40 mA
Power	10 W	22 W
Time	30 min	115 min
Actual start voltage (appr.)	150 V	340 V
Temperature	15°C	
For a half gel: halve mA and W, do not change V !		

- Switch on the Cooling Unit, adjust to 15 °C.

6.7 Finishing the Electrophoresis

- Finishing the electrophoresis: For maximum separation capacity the bromphenolblue front should enter the anodal electrode strip. If not, continue the run until the bromphenolblue front reaches the anodal strip (check regularly).

Note: Running time may vary due to different sample properties.

- Dispose the electrode strips.
- After electrophoresis remove the gel from the electrophoresis unit and rinse the gel using ddH₂O to remove Cooling Solution. Proceed to Post-electrophoretic application (Staining, Imaging, Blotting etc.).
- Clean the Cooling plate of the horizontal electrophoresis system and the electrodes with ddH₂O. For cleaning use lintfree tissues.

7 Fixation and Storage of the gel

Fixate proteins in the gel for 30 min using 20% (v/v) Ethanol and 7% (v/v) Acetic Acid. Store gels in 20% (v/v) Ethanol and 3% (v/v) Glycerol.

8 Staining / Imaging / Blotting

Product No.	Gel	suitable for fluorescence detection	suitable for Coomassie staining	suitable for silver staining
PR 254	VELUM GOLD Precast 1D Gels	blue, green, red and infrared	cold	no
PR 255	VELUM GOLD Precast 1D Gels	blue, green, red and infrared	cold	no
PR 256	VELUM SILVER Precast 1D Gels	red and infrared	cold + hot	yes
PR 257	VELUM SILVER Precast 1D Gels	red and infrared	cold + hot	yes

Do not heat VELUM Precast Gels.

Avoid concentrations of organic solvents above 40%(v/v).

For Blotting of the VELUM Precast Gels use the BEO or VELUM Dry Blotter (PR87 und PR88).