NH DyeAGNOSTICS

Product Guide

VELUM Precast 2D Gels

Product no. PR221, PR222, PR237, PR241

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For research use only

1 Kit content

- 4 VELUM Precast 2D Gels (12,5 % AA/BisAA)
- Cathode Strip (colorless)
- Anode Strip (blue)
- VELUM Precast 2D Gel Equilibration Buffer
- VELUM Precast Gel Cooling Solution

2 Additional material required (not included)

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

3 Storage and stability

See package label

4 Gel types

Product no.	Gel	size	slots / IPG strips	suitable for fluorescence detection
PR 241	VELUM GOLD Precast 2D Gels large	250 x 180 x 0.5 mm	1 slot (for one 18 - 24 cm IPG strip) plus marker slot	blue, green, red and infrared
PR 222	VELUM SILVER Precast 2D Gels large	250 x 180 x 0.5 mm	1 slot (for one 18 - 24 cm IPG strip) plus marker slot	red and infrared
PR 237	VELUM GOLD Precast 2D Gels small	250 x 110 x 0.5 mm	2 slots (for one 7-11 cm IPG strip each) plus marker slot	blue, green, red and infrared
PR 221	VELUM SILVER Precast 2D Gels small	250 x 110 x 0.5 mm	2 slots (for one 7-11 cm IPG strip each) plus marker slot	red and infrared

5 General information

VELUM Precast 2D 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 2D Gels are low fluorescent and therefore ideal for fluorescent labelling and staining in a spectrum from blue to infrared and also for common Coomassie and SILVER staining.

6 Instructions for use

6.1 Preparation of the horizontal electrophoresis system

- Clean the cooling plate and the electrodes with ddH_2O using a lintfree tissue.
- Apply 2 ml VELUM Precast Gel Cooling Solution onto the cooling plate along the center line.

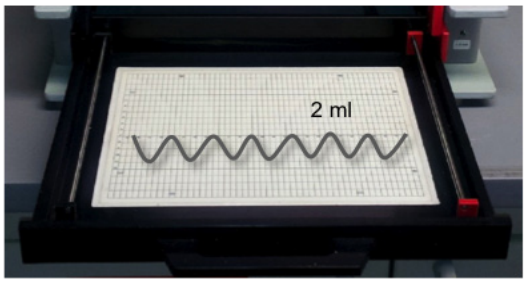

Fig. 2: Application of the Cooling Solution

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 strip

- Place the Anode Strip onto the anodal edge (far from wells). Wick-gel-overlap: 5mm
- Place the Cathode Strip onto the cathodal edge (sample side). Wick-gel-overlap: 5mm

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

6.4 Equilibration and Placing of the IPG-Strips

6.4.1 Preparation of the Equilibration solutions:

You will need 3 equilibration solutions. 5 ml of each solution per IPG-Strip. Prepare the solution freshly before use as follws:

5 ml Equilibration solution R (reduction of disulfide bridges):

1,5 g Urea
+ 40 mg DTT
+ 4,2 ml VELUM Precast 2D Gel Equilibration Buffer

5 ml Equilibration solution A (alkylation of reduced SH-groups):

1,5 g Urea
+ 105 mg IAA
+ 4,2 ml VELUM Precast 2D Gel Equilibration Buffer

5 ml Equilibration solution F (removal of Equilibration solutions R and A):

1,5 g Urea
+ 4,2 ml VELUM Precast 2D Gel Equilibration Buffer

Make sure that all solutions are well mixed and all solid components arre completely solved.

Caution: Do not warm up Urea containing solutions over 30 °C.

6.4.2 Equilibration of the IPG-Strip:

Inkubate the IPG-Strip on a shaker in the Equilibration solution as follows:

1. 15 min in Equilibration solution R,
2. 13 min in Equilibration solution A and
3. 2 min in Equilibration solution F.

Remove the excess of equilibration solution on the back of the IPG-Strip using lintfree tissue and let the remaining solution drain in a lintfree tissue.

6.4.3 Placing the IPG-Strip:

- Cut the film support of the IPG-Strip, so that there are max. 2 mm of film support left on both sides of the strip.
- Place the IPG-Strip in the well by using 2 tweezers. Gel side of the IPG-Strip downwards (towards the 2D gel; fig. 3).
- Ensure proper IPG-Strip-gel-contact by pressing the IPG-Strip on the anodal side of the well.

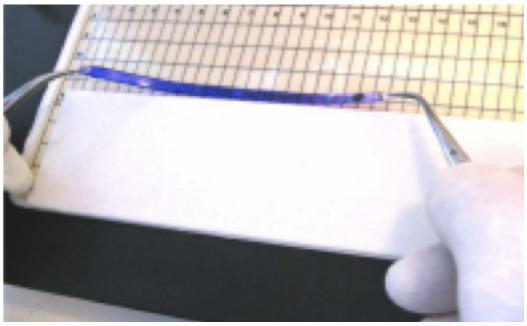
Fig. 3: Placing the IPG-Strip

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 Cathode Strip (colorless, sample side) and the positive voltage (+) onto the Anode 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 (Cathode Strip at sample side, Anode Strip at sample far side).

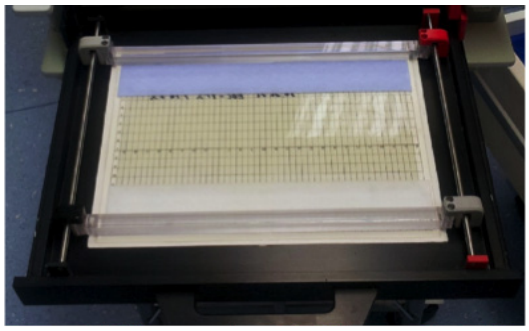

Fig. 4: Arrangement of gel, wicks and electrodes

6.7 Gel electrophoresis

- Switch on the Cooling Unit, adjusted to 15 °C.
- Start the gel electrophoresis. You can choose between 2 different protocols, a day-run (about 5 h) and an over-night-run (about 19 h).
- use the following parameters for the run of one gel:

  Note: Choose for day run (ca. 4-5 h) and over night run (ca. 19 h). Electrophoretic parameters and settings depend on gel size (different for small and large gels)

VELUM GOLD/SILVER Precast 2D Gels large (for 18-24 cm IPG strips; PR241, PR222): short run (ca. 5 h)				
Parameter	**Step 1**	**Step 2**	**Step 3**	**Step 4**
Voltage	100 V	200 V	300 V	800 V
Current	12 mA	20 mA	25 mA	40 mA
Power	2 W	5 W	10 W	30 W
Time	30 min	20 min	10 min	170 min
Actual start voltage (appr.)	80 V	160 V	230 V	380 V
Temperature	15°C			
Caution: Remove the IPG-Strip after step 3 !				

VELUM GOLD/SILVER Precast 2D Gels large (for 18-24 cm IPG strips; PR241, PR222): over night run (ca. 19 h)					
Parameter	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Step 5**
Voltage	100 V	200 V	300 V	150 V	1000 V
Current	12 mA	20 mA	25 mA	7 mA	25 mA
Power	2 W	5 W	10 W	2 W	25 W
Time	30 min	20 min	10 min	17 h	60 min
Actual start voltage (appr.)	80 V	160 V	230 V	90 V	820 V
Temperature	15°C				
Caution: Remove the IPG-Strip after step 3 !					

VELUM GOLD/SILVER Precast 2D Gels small (for 7-11 cm IPG strips; PR237, PR221): short run (ca. 3 h)				
Parameter	**Step 1**	**Step 2**	**Step 3**	**Step 4**
Voltage	100 V	150 V	200 V	1000 V
Current	7 mA	13 mA	20 mA	35 mA
Power	2 W	3 W	5 W	25 W
Time	30 min	30 min	10 min	110 min
Actual start voltage (appr.)	40 V	90 V	160 V	310 V
Temperature	15°C			
Caution: Remove the IPG-Strip after step 3 !				

VELUM GOLD/SILVER Precast 2D Gels small (for 7-11 cm IPG strips; PR237, PR221): over night run (ca. 19 h)				
Parameter	**Step 1**	**Step 2**	**Step 3**	**Step 4**
Voltage	100 V	150 V	250 V	100 V
Current	7 mA	13 mA	20 mA	6 mA
Power	2 W	3 W	5 W	2 W
Time	30 min	30 min	10 min	17 h
Actual start voltage (appr.)	50 V	90 V	150 V	100 V
Temperature	15°C			
Caution: Remove the IPG-Strip after step 3 !				

- Finishing the electrophoresis: For maximum separation capacity stop electrophoresis when the bromphenolblue front enters the anodal electrode wick.

  Note: Run 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 ddH_2O 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 ddH_2O. For cleaning use lintfree tissues.

6.9 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.

6.10 Staining / Imaging / Blotting

Product No.	Gel	suitable for fluores-cence detection	suitable for Coo-massie staining	suitable for silver staining
PR 241	VELUM GOLD Precast 2D Gels large	blue, green, red and infrared	cold	no
PR 222	VELUM SILVER Precast 2D Gels large	red and infrared	cold + hot	yes
PR 237	VELUM GOLD Precast 2D Gels small	blue, green, red and infrared	cold	no
PR 221	VELUM SILVER Precast 2D Gels small	red and infrared	cold + hot	yes

Do not heat VELUM GOLD Precast Gels.

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

For Blotting of the VELUM Precast Gel use the BEO or VELUM Dry Blotter (PR 87 und PR88).