Running 2DGel DALT in DALT*twelve*

Always:

- **Wear powder-free disposable gloves.**
- **Do not touch or contaminate the gel surface.**
- **Only handle gel by the film margins.**
- **Store gels in a refrigerator but do not freeze.**
- **Only use gelcompany buffer kit and equilibration solutions.**

Loading the FlapCassette

1. First load the FlapCassette (gelcompany cat # 1004-40; protocol P-1003-40 for details.)
2. Pre-cool FlapCassettes in a refrigerator for at least 15 min before inserting the IPG-strips. This will assist the setting of the LM-agarose.

Prepare the running buffers:

3. Anode *(lower) buffer:* Dissolve the content of the anode buffer bag in 7.5 L H_2O_{dist}.
4. *Cathode (upper) buffer:* Dissolve the content of the cathode buffer bag in 2.5 L H_2O_{dist}.
5. Close the draining valve, pour 7.5 L anode buffer into the lower buffer tank. Switch the pump on and set the temperature to 25 °C.

Agarose imbedding of the IPG strips:

Caution: Temperatures above 45°C may damage the plastic backing of the gel.

6. Melt the LM-agarose in a 70°C water bath and maintain molten at 40°C (water adjusted to the correct temperature will surfice).
7. Pipette 1 mL of 40°C agarose into a microcentrifuge tube (1.5 mL) and cool it under tap water (3 sec), then quickly pipette 30 µL into each of the top corners of the cassette to close the gaps between the gel and spacers (fig 1a).
8. Pipette 5 mL of 40°C LM-agarose into the cassette, insert the IPG strip with the film-backing towards the glass plate, and push it down to the gel edge with the IPG-StripPusher (fig. 1b; gelcompany cat # 1003-55).

Caution: Do not push too hard to avoid damage of the gel edge.

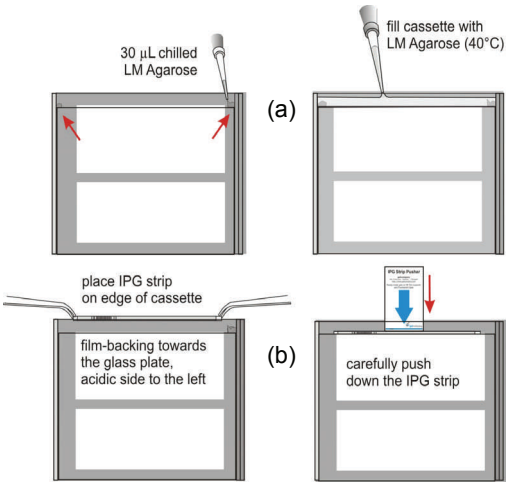

Fig. 1

Loading the DALTtwelve

9. Place all gel cassettes and blank cassettes into the sealing manifold of the Ettan DALT*twelve* (fig.2). Take care that the silicone rubber sealings do not bend downwards.

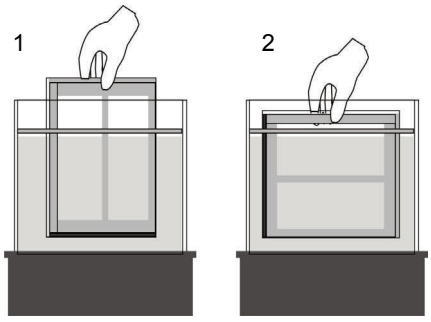

Fig. 2.

10. Make sure, that the liquid level of the lower buffer tank is filled to about 3 mm below the tubing of the sealing manifold. If necessary add distilled water to the lower buffer to reach this level.

Note: FlapCassettes 2D displace much less anodal buffer volume than blank cassettes (ca. 100 mL less per cassette).

Note: When filling level of the lower buffer tank it is necessary to achieve a hydrostatic balance, to prevent leakage of the upper buffer and mixing of anodal and cathodal buffer.

11. Carefully pour the 2.5 L upper buffer into the upper buffer chamber.

Running the Electrophoresis

12. Close the lid, switch on the thermostatic circulator (25°C) and start the run (It must be run with constant power [W] to prevent overheating of the buffer) according to the tables on the second page.

Note: If you have trouble sealing with the LM-agarose refrigerate for longer or at a lower temperature. "Chilling sprays" can be applied locally to facilitate setting of LM-agarose.

This protocol is forms part of a series that describe the use of gelcompany products in the 2D-gel workflow. For other protocols visit our website.

Running conditions: See the tables for the maximum settings.

Note: *The achieved voltage and the running times can vary dependent on the conductivity of the distilled or deionzed water used for dissolving the buffer powder. The use of homogeneous or gradient gels can influence the voltage and running time.*

The specifications “const“ (constant) and “limit“ are added for programming the Bio-Rad power pack.

mA and voltage values are :

Tab. 1: Running conditions for day run (25 °C)

Steps	V / mA	Constant Power (W)												Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***5 gels***	***6 gels***	***7 gels***	***8 gels***	***9 gels***	***10 gels***	***11 gels***	***12 gels***	
S1	open	1	1	2	2	3	3	4	4	5	5	6	6	1 h
S2	open	15	30	45	60	75	90	105	120	135	150	165	180	6 h

Tab. 2: Running conditions over night (25 °C)

Steps	V / mA	Constant Power (W)												Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***5 gels***	***6 gels***	***7 gels***	***8 gels***	***9 gels***	***10 gels***	***11 gels***	***12 gels***	
S1	open	1	1	2	2	3	3	4	4	5	5	6	6	2 h
S2	open	2	5	7	10	12	15	17	20	22	25	27	30	14 h