Running 2DGel DALT in DALT*six*

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 (**gel**company 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 buffer: Dissolve the anode buffer bag contents in 4.5L $H_2O_{dist.}$ and pour into the lower buffer tank. Plug-in pump cable.
4. Cathode (upper) buffer: Dissolve the content of the cathode buffer bag in 1.4 L $H_2O_{dist.}$

Agarose imbedding of the IPG strips:

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

5. Melt the LM-agarose in a 70°C water bath and then maintain molten in a 40°C waterbath (beakers containing water adjusted to the correct temperature will surfice).
6. Pipette 1 mL of 40°C agarose into a microcentrifuge tube (1.5 mL) and cool it under the tap (3 sec) then quickly pipette 30 µL agarose into the top corners of the cassette to close the gaps between gel and spacers (fig. 1a). If the gap does not seal repeat this operation.
7. Pipette 5 mL of 40°C LM-agarose into the cassette. Insert the IPG strip, film-backing towards the glass plate, gently push it down to the gel edge with the IPG-StripPusher (fig. 1b; **gel**company cat # 1003-55).

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

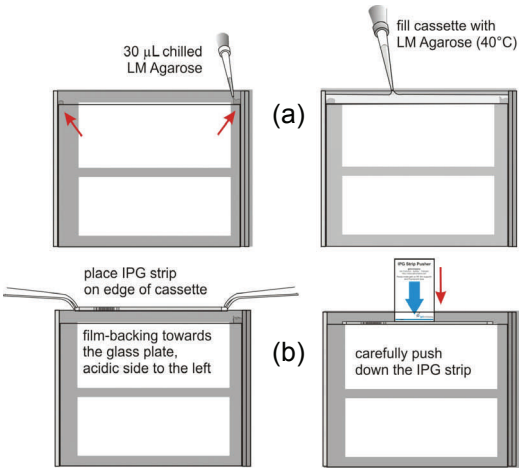

Fig. 1

Loading the DALTsix

8. After all gel cassettes and blank cassettes are inserted into the rack, spray the upper buffer chamber with 0.1 % SDS water and apply it onto the cassettes and the rack.
9. Place the rack carefully into the lower buffer chamber (avoiding air bubbles). Then carefully pour the 1.4 L upper buffer into the upper buffer chamber.
10. Immediately fill the lower buffer tank with distilled water to the same filling level as in the upper buffer (fig. 2).

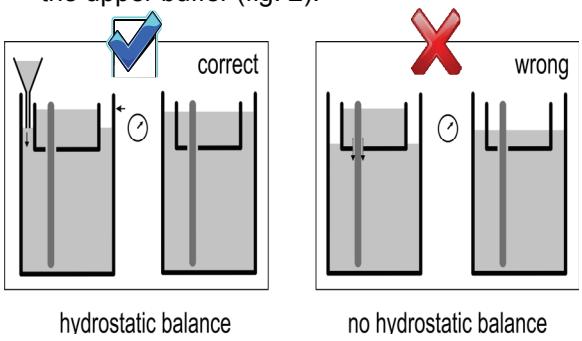

Fig. 2

Caution: When filling 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.

Note: FlapCassette 2D displaces approximately 100 mL less anodal buffer volume than blank cassettes.

Running the Electrophoresis.

11. 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.
12. Remove the adhesive tape from the gel before staining.

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

1. **Running conditions:** See the tables for the maximum settings.
2. ***Note:*** *The achieved voltage and the running times can vary dependent on the conductivity of the distilled or deionized water used for dissolving the buffer powder. Also the use of homogeneous or gradient gels influence the voltage and time.*
3. The specifications “const“ (constant) and “limit“ are added for programming the Bio-Rad power pack.
4. mA and voltage values are :

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

Steps	Voltage	Current [mA]						Power (W)						Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***5 gels***	***6 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	***5 gels***	***6 gels***	
S1	50 V limit	10 limit	20 limit	30 limit	40 limit	50 limit	60 limit	1 const	1 const	2 const	2 const	3 const	3 const	1 h
S2	600 V limit	60 limit	120 limit	180 limit	240 limit	300 limit	360 limit	30 const	60 const	90 const	120 const	150 const	180 const	5 h

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

Steps	Voltage	Current [mA]						Power (W)						Time
		1 gel	***2 gels***	***3 gels***	***4 gels***	***5 gels***	***6 gels***	***1 gel***	***2 gels***	***3 gels***	***4 gels***	***5 gels***	***6 gels***	
S1	50 V limit	10 limit	20 limit	30 limit	40 limit	50 limit	60 limit	1 const	1 const	2 const	2 const	3 const	3 const	1 h
S2	250 V limit	30 limit	60 limit	90 limit	120 limit	150 limit	180 limit	2.5 const	5 const	7.5 const	10 const	12.5 const	15 const	14 h