

**DGGE – preparation of gels****ML03.001.001**

Responsible: CSJ

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**Preparation of gels**

Cautions: Wear gloves all times when handling acryl amide and form amide solutions and all work with acryl amide and form amide has to be done in a flow hood. Acryl amide is very toxic. After the gels have polymerized the toxicity does decrease significantly and the gels can be handled outside the flowhood.

The table gives instructions for acryl amid solutions (total volume 100ml) for use in preparation of DGGE gels (8% acryl amid gels). For preparation of acryl amid gels with different acryl amid percentages ref. e.g. the DGGE manual (*The Dcode Universal Mutation Detection System*).

	0%	10%	15%	20%	25%	30%	35%	40%
40% acryl amid/Bis (ml)	20	20	20	20	20	20	20	20
50x TAE (ml)	2	2	2	2	2	2	2	2
Form amid (deionised, ml)	0	4	6	8	10	12	14	16
Urea (g)	0	4,2	6,3	8,4	10,5	12,6	14,7	16,8
dH <sub>2</sub> O (MilliQ, ml)	78	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100
	45%	50%	55%	60%	70%	80%	90%	100%
40% acryl amid/Bis (ml)	20	20	20	20	20	20	20	20
50x TAE (ml)	2	2	2	2	2	2	2	2
Form amid (deionised, ml)	18	20	22	24	28	32	36	40
Urea (g)	18,9	21,0	23,1	25,2	29,4	33,6	37,8	42,0
dH <sub>2</sub> O (MilliQ, ml)	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

\* the table presents how to prepare acryl amid solutions with varying denaturation percentage (%)

**Preparation of deionised form amid (500ml)**

- 500ml formamid and 25g resin (AG<sup>®</sup>501-X8 (D), BioRad) are transferred to a 1 litre Red cap flask.
- The flask is placed on a horizontally shaking table (fasten safely) and shaken for 1 hour at 150rpm. When the resin beads change colour from blue to yellow the deionisation is taking

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place. After 1 hour shaking blue resin beads should still be present in the solution (to make sure a sufficient deionisation capacity is present).

- The formamid solution is calmly filtered through filter paper (cut “elephant” paper into a proper size) into a brown 500ml glass flask. The form amid solution should be kept in the fridge at 5°C (it has a long expiration, app. 1 year).

**Preparation of acryl amid solutions**

- A brown flask is added in the amount as written in the table above (You can also cover a colourless bottle with alu-foil). The solutions/chemicals should be added in the order as mentioned in the table (from the top, always starting with the acryl amide and so forth downwards). It is important that MilliQ is added last since urea makes up a large volume addition.
- The acryl amid solution is shaken for 10 min (150rpm) followed by degassing (in the ultra sound bath) for another 10 min. If not the urea has been fully dissolved a slight heating under the warm tap water might help (**Warning:** never heat acryl amide solutions above 30 °C outside a hood). It is recommended not to make larger acryl amid solutions than 100ml! The acryl amid solutions have a short lifetime (expires 2-4 weeks after preparation).
- After usage the remainder of the acryl amid solution should be transferred to 50ml NUNC single use tubes. The brown flask is then washed 3 times with water and the washing water (containing what is left of acryl amid in the flask) is also collected in 50ml NUNC tubes. The NUNC tubes are put in a plastic bag and discarded in blue barrel marked “DGGE/ethidiumbromid affald” (it is placed in the wasteshed in the yard).

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## How to run DGGE

### Assembling the glass plates

- Wipe of two glass plates with 96% ethanol, 1 big and 1 small per gel. The plates should be completely clean and free of old gel remains.
- Assemble the plates on the stand with two 1,0mm (or e.g. 0,75mm) spacers in the intended holders.
- Check that the glass plates are correctly assembled with the adjustment card
- If only 1 gel is prepared, still 2 plates must be assembled. The other set of plates then just has to be with out spacers.
- The glass plates are placed in “casting stand” – on top of the grey rubber pieces and tightened

### Cast of gel

- Degas the acryl amid solutions for 10 min in the ultra sound bath
- HERE AFTER ALL WORK IS PERFORMED IN THE FUMEHOOD! (Non-polymerised acryl amid is carcinogenic!!)
- Get all laboratory equipment ready (pump, magnetic stirrer, gradient mixer, tube, magnets, pipettes, tips, ammoniumpersulfat (Ammoniumperoxodisulfat,  $(\text{NH}_4)_2\text{S}_2\text{O}_8$ ), TEMED, wash bottle with MilliQ, glass container for waste), and assemble the gel equipment.
- The syringe needle is fastened at the “glass sandwich” opening and the pump is adjusted to a speed of 2,5 (equal to 10 ml MilliQ in 43 seconds). A magnet is placed in each chimney.
- Carefully add (without blowing any air into the solution)  $2 \times 18$  ml degassed acryl amid solution to the gradient mixer – **low** and **high** denaturation concentration in a chimney each. **High** should in the chimney **nearest** to the tube outlet.
- Start the magnetic stirrer
- Add 135 $\mu$ l ammoniumpersulfate (10% wt/vol, can be freezed in small eppendorpf tubes and last for one month) to each chimney and thereafter add 13,5 $\mu$ l TEMED. The chemical reaction now starts and the acryl amid solution polymerises. The solutions are mixed by stirring for app. 15 sec.

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- Quickly do the following in the mentioned order:
  1. Open for the tube
  2. Turn on the pump
  3. Open the mixing valve
- The glass sandwich is then filled with the gel material (all the way up to a few mm from the upper edge) and a 1mm comb is placed between the plates from the top. Excess gel material will then overflow but it is important to fill the glass sandwich that much since the gel shrinks when it dries. The gel is placed to dry for at least 4 hours (and much rather for a longer time)
- Right after the gel has been cast the two chimneys should be cleaned with MilliQ. Wash 3 times. Waste water from the washing is collected in 50ml NUNC tubes and discarded in the blue barrel marked “DGGE/ethidiumbromid affald” (It is VERY important to do the washing right after finishing the cast since small remainders of gel material get stocked in the system and block the passage!!!)
- Excess of water in the system after washing is tapped out over a tissue and the two chimneys are blown through with air. The equipment is then ready for cast of the second gel or can be put away.

**Loading of the gel**

- The comb is carefully removed when the gel has solidified.
- NOW THE REST OF THE WORK CAN BE DONE OUTSIDE THE FUMEHOOD – since the acryl amid has polymerised!
- Make a 1×TAE buffer solution from a 50×TAE buffer solution (can be found in the gel room). Take 100ml 50×TAE and transfer it to a 5L flask and add MilliQ up to 5L.
- Fill up to the electrophoreses chamber (to right under the FILL-mark) with the 1×TAE dilution
- The gel is placed in the holder with the smallest glass plate facing towards the middle of the holder. Place the holder with the gels in the chamber with the red dot facing the right side of the chamber.

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- Fill the chamber in between the two glass plates with 1×TAE dilution and heat the vessel up to 60°C (takes app. 1 hour).
- The 1×TAE dilution should be at the RUN marked level. Check the liquid level between the two glass plates has not dropped during heating. If the liquid level has dropped the plates are not properly fixed in the holder.
- The PCR product is mixed with loading buffer and the samples are then loaded on the gel.
- The electric voltages are set to 70V and the gel is run for 17 hours.

**Staining the gel**

- After 17 hours, the instrument is turned off and the holder with the gels is removed from the chamber
- Use the spacers to carefully lift off the smallest glass plate
- Prepare a gel dye solution by mixing 2µl SYBR<sup>®</sup>GOLD and 20ml 1×TAE buffer
- Transfer the plate with the gel to a squared plastic container with a lid and distribute the dye solution evenly over the gel.
- Leave the gel in the dark for 45min
- Rinse the gel for excess dye solution with MilliQ water
- Transfer the gel to the “Gel-documentation system” up side down and take a nice picture ☺
- The gel is put in a plastic bag and disposed in the blue barrel marked “DGGE/ethidiumbromid affald”
- The glass plates thoroughly cleaned with MilliQ water and following wiped of with 96% ethanol.