Poly-Acrylamide Gel Substrate Protocol
Fredberg Lab
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1. Silane Treatment for Acrylamide-Glass Binding

If not on-hand, prepare silane binder stock:
Mix 200ml DI water + 80µl acetic acid glacial + 50µl silane agent Silane agent stored in 4°C fridge: 3-(Trimethoxysilyl) poropylmethacrylate.

1. Pipette ~1 ml of bind silane mixture onto glass-bottom petri dish.

2. Cover with lid and incubate for **1 hour** at room temp.

3. During incubation, begin preparation of acrylamide gel mixture and start degassing.

4. After 1hr incubation, thoroughly wash glass-bottom dishes with DI water to remove excess silane binder.

5. Dry glass-bottom slides with compressed air gun.

2. Prepare Acrylamide Gel Mixture

1. Mix acrylamide, bisacrylamide, TEMED, bead, and water in a 1.5ml Eppendorf tube according to recipe given in the table (below) for the desired gel stiffness.

2. De-gas acrylamide mixture (in vacuum chamber) **for at least 30min**.

3. In a separate Eppendorf tube, prepare Ammonium Persulfate (APS) stock (see below table).

4. Pause here until bind silane incubation is complete and petri dishes are washed and dried.

3. Begin Gel Polymerization

1. Add the APS polymerizing agents into the acrylamide mixture. While adding the APS, convectively mix the mixture by numerous pipetting.

2. DO NOT vortex the final mixture as this will introduce oxygen and delay acrylamide polymerization.

3. Polymerization begins immediately after adding the APS to the acrylamide mixture. The next steps should be done as quickly as possible. If using beads, attempts should be made to ensure that bead sedimentation centrifuging (next step) starts no later than 4-5 minutes. Catalyst volume should be adjusted to slow polymerization if using centrifuge and beads.

4. Pipette the actively polymerizing gel onto center of glass-bottom dish. The volume pipetted will determine the thickness of the gel.

   For a thin gel: 24 µl = 100 µm: This is the one we use.
   For a thick gel: 500 µl = 1 mm

5. Immediately after pipetting, place a round glass coverslip over the gel droplet to spread the gel across the bottom-glass slip. The cover slip thickness changes with gel thickness.

   For a thin gel: 18 mm: This is the one we use.
   For a thick gel: 25 mm

6. Cover the dish with the lid and invert so that it is upside down.

4. Sediment beads to top focal plane for traction microscopy

1. Once all glass-bottom dishes have been prepared, stack dishes upside down in two equal towers. Tape together each tower and place in swinging bucket centrifuge rotor.

Spin at:
For a thin gel: 500 rpm for 8 min
For a thick gel: 600 rpm for 20 min

2. Remove gels from centrifuge and place in degasser if further polymerization is required. Time varies with rigidity/amount of cross-linkers. Expected time is around 25-35 minutes.

3. Two alternatives:

   a. Leave out at room temp to allow polymerization to proceed – will take substantially longer. Ensure that gel does not dehydrate whereby ripples are created.
   b. Put in nitrogen chamber to ensure that the amount of oxygen is reduced.

4. Continuously check gel's appearance.

5. Once sufficient gel polymerization has occurred, submerge gel in DI water and place in 4°C fridge until needed.

6. Store for up to 3 days. (Emily / Chan do not limit the storage time even after the coverslip has been removed)

7. Prior to use – remove coverslip.

8. Proceed to SANPAH and Collagen protocol.

5. References

The two original references are that of Engler et al. (*1*) and Yueng et al. (*2*). A new paper reconfirms their results (*3*) . Perhaps, the paper with the easiest instructions is Ref. (*2*).

1. A. Engler *et al.*, Substrate Compliance versus Ligand Density in Cell on Gel Responses. *Biophys. J.* **86**, 617–628 (2004).

2. T. Yeung *et al.*, Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motil. Cytoskeleton*. **60**, 24–34 (2005).

3. J. D. Mih *et al.*, A Multiwell Platform for Studying Stiffness-Dependent Cell Biology. *PLOS ONE*. **6**, e19929 (2011).

Acrylamide Gel Recipe Look-Up Table

E (Pa)	150	300	600	1200	2400	4800	9600	19200	38400	76800	153600
G (Pa)	50	100	200	400	800	1600	3200	6400	12800	25600	51200
Acrylamide (%)	3	3	3	3	7.5	7.5	7.5	7.5	12	12	12
Bisacrylamide (%)	0.04	0.048	0.058	0.108	0.034	0.053	0.148	0.236	0.118	0.242	0.585
Mixture (µl)											
40% Acrylamide	75	75	75	75	188	188	188	188	300	300	300
2% Bisacrylamide	20	24	29	54	17	27	59	118	59	121	295
0.5 µm Beads	60	60	60	60	60	60	60	60	60	60	60
Ultrapure H2O	745	740	735	710	635	625	590	535	480	420	245
TEMED	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
APS Stock (1-5%)*	100	100	100	100	100	100	100	100	100	100	100
Final Volume	1000.5	999.5	999.5	999.5	1000.5	1000.5	997.5	1001.5	999.5	1001.5	1000.5

TEMED Preparation alternative:
Prepare a 10fold dilution of TEMED, and use 10X as much in the acrylamide gel mixture.
ie. TEMED dilution = 5 ul TEMED + 45 ul H2O. Then use 5 ul TEMED dilution in acrylamide gel mixture.
Adjust total volume by subtracting 4.5 ul of water.

APS Stock:
1% = 10mg APS + 1ml DI water.
5% = 50mg APS + 1ml DI water.

*Softer gels require the higher APS concentration.