

POLYACRYLAMIDE GEL FABRICATION FLOWCHART

Activate coverslips

1. Silanize with **3-aminopropyl-trimethoxysilane** (2 hours + 30 minutes waiting to cool off)

First turn the oven on, HIGH setting, @ 160°C

Wipe and air-blow each square (22mm, No.1) coverslip and put on “silanized” rack

Then follow “**Glass coverslip silanization**” protocol

Store in “silanized” desiccator

2. Activate with **glutaraldehyde** (1 hour + 15 minutes transferring to “dry only” racks)

Make sure coverslips cooled after silanization before starting

Move all coverslips from “silanized” racks to “glutaraldehyzed” racks

Then follow “**Activation with glutaraldehyde**” protocol

Store in “silanized+glutaraldehyzed” desiccator

Never mix up racks from different steps, or you’ll get strange reactions resulting in reddish-stained coverslips that don’t bind gel properly.

3. Make polyacrylamide gels (3 hours)

Choose appropriate gel for your cells and printout the titration table:

Stiff: 6% acrylamide (MDCK)
0.15% BIS

Medium: 5% acrylamide (C3H, MEF, CHO, NBT-II)
0.1% BIS

Soft: 4.5% acrylamide (keratocytes, NBT-II)
0.07% BIS

Follow the “**Polyacrylamide gel fabrication**” protocol.

Make sure you wash gels properly, discard all that don’t look like they x-linked properly. If you want particular gel thickness, use spacers to support the sandwich.

4. Activate gel (min 6 hours)

Follow “**Activation with hydrazine hydrate**” protocol, make sure they’re washed properly.

Can keep in hydrazine overnight (after 22 hours gels were still fine).

Store each gel in a 35mm dish @4°C in PBS (max 2 months, just replenish evaporated PBS).

5. Conjugate collagen (or other ECM protein) to activated gel (min 6 hours)

Make sure you do all that is possible under the cell culture hood.

Follow “**Collagen conjugation**” protocol and make sure to do washes properly.

Store at @4°C in PBS for up to 5 days.

Storing

Silane, TEMED, ammonium persulfate, sodium periodate → store in desiccator

Hydrazine hydrate → store under the fume hood, in secondary confinement

Glutaraldehyde → store @4°C

Waste → both hydrazine and silane (alkylating) waste bottles must be in secondary confinement

Collagen → 1mg/ml stock @4°C for up to 1 week, @-78°C long-term

Testing chamber → in plastic dish, after being washed in mild detergent, rinsed with H₂O^{dd} and dried with kimwipe.

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