SANPAH & Collagen Coating Protocol
Fredberg Lab
02-02-2018

.

SANPAH aliquot preparation:

Take 100 mg bottle of SANPAH (located where?) and directly add 2 ml DMSO. Mix.
Aliquot 40 µl into black, opaque screw-cap tubes. This is @ 50 mg/mL.
Place in Freezer A

50 mM HEPES Buffer preparation:
Add 25 mL of 1M HEPES directly to 500 mL PBS bottle. (Bottle of 1M HEPES solution is stored in fridge B.)

1. Apply SANPAH to acrylamide gel

1. *Thaw SANPAH aliquot and prepare dilution right before needed.

2. To remove the SANPAH, add 1 ml of the HEPES directly to the SANPAH aliquot. Pipet fluid and place in large vial. Rinse vial with additional 1 ml of HEPES.

3. Add another 2 mL of HEPES buffer (for a total of 4 ml). Mix.

4. Pipette ~440 µL of SANPAH dilution directly onto the gel surface.

5. Transfer gels to UV lamp station located in inner lab. Expose the gel (with SANPAH) for a total of 10 minutes. Rotate gels half-way through exposure.

6. Wash gels 3x with HEPES (fill entire dish each time).

7. Then wash gels 3x with PBS buffer. Leave the last PBS wash in the dish so gel does not dry before next step.

2 Apply Collagen to SANPAH coated Acrylamide

1. Bovine Collagen-1 is located in fridge A. Stock solution is usually ~3.2 mg/mL (double check)

2. Prepare Collagen-1 solution at 0.1 mg/mL in HEPES buffer.
(Karin uses 50 µg/mL and Jae uses 20 µg/mL)

(Need 1 ml to cover each gel for 12-well plates. Need ~500uL for single glass-bottom dish. However, while filtering the collagen, some of it is lost in the filter. So plan dilution and total volume accordingly.)

3. Filter 0.1 mg/mL Collagen-1 solution with 0.2 μm filter.

4. Immediately after filtering, pipette 1 ml (or other required amount) of Collagen-1 solution onto each gel.

5. Cover gels with dish lids and place in fridge.

6. Leave overnight at 4C.

The next day:

7. Aspirate and rinse with HEPES.

8. Leave collagen coated acrylamide gels in HEPES until needed. Use within 3 days.

9. Before use, store at room temp or in incubator to accommodate cells.