**Protein Gel - SDS Page**

* Upper Gel- makes the proteins enter the gel in a uniform way.
* Lower Gel- the running gel itself.
* We normally use a 1mm glass.
* We use a denture gel- SDS page to open and negatively charge the proteins, DTT to break sulfur bonds.

**Protocol:**

1. **Prepare the gel protein device, test it for leaking** : place the device on a flat surface, wet the glasses, and place them together, open the 2 green windows at once. Attach to the colorless plastic device using the clamp, lower the green sides at once. Add water up until the end, and make sure there is no leaking.
2. **Gel preparation:**
   1. **Lower gel**: mix by this order the following materials (quantities are for 1 1mm glass):
      1. DIW- 3 ml
      2. Acrylamide - 2.5 ml (old fridge bottom shelf brown sigma bottle) - **in hood**
      3. 1.5M Tris 8.8- 1.9 ml (Shahar's bench- on shelf)
      4. 10% SDS - 75 ul (Shahar's bench)
      5. 10% APS - 75 ul (in the new freezer- upper shelf white box)
      6. TEMED - 3 ul (under the hood) - **in hood**

Place the lower gel in the device (after removing the water) up to the upper green level. Add a bit od DIW to straighten the gel's level (you can also use IPA 10% for max precision). Wait for the gel to harden (10-40 min').

1. **Upper Gel**: mix by this order the following materials (quantities are for 1 1mm glass):
   1. DIW- 2 ml
   2. Acrylamide - 500 ul
   3. 1M Tris 6.8- 375 ul (Shahar's bench- on shelf)
   4. 10% SDS - 30 ul
   5. 10% APS - 30 ul
   6. TEMED - 3 ul (under the hood) - **in hood**

Once the lower gel has hardened, remove DIW and add the upper gel. Insert the comb to create wells- up until the lower gel level.

1. **Sample preparation**: in each sample, place 7ul DIW, 2ul sample buffer (in the new fridge, upper shelf's stand), 1ul of the sample. The sample buffer itself contains DTT and SDS. Place the samples in boiled water for 5 minutes, to maximize denaturation.
2. **Assembling the running device**: if one gel is used, place a thick 1mm glass in one side of the running device, place your gel (once removed from the preparation device) in the other side (the thick glass is facing to you), to create a sealed chamber. Place in the running tank, add the running buffer (over Shahar's bench, X5- dilute to X1). First fill the chamber, then fill the tank itself.
3. **Loading the samples**: use a narrow tip to load the samples- always take more volume then needed into the tip- so you can see the air bubbles coming out once you are in the well. The marker is placed in the old freezer, on the blue stand- PageRuler.
4. **Running the gel:** 20-25 mA, until the marker is opened, then up to 120V.
5. **Removing the gel:** use the green plastic putty to extract the gel from the glasses, place it into a small flat surface plastic box, add DIW.
6. **Results**: Wash the gel using DIW: place DIW into the box until it is covering the gel, 50 rpm 8 min', repeat this action 3 times. Add the coloring Dye- **Fast SeeBand**- in the old fridge lower shelf. 20 min' up to O.N.