Important: Protamines are very basic proteins due to their having a high percentage of arginines in their amino acid sequences. Protamines are insoluble in SDS and cannot be run on standard SDS gels.

Acid Urea Slab Gels

Stock solutions: Caution: wear gloves, acrylamide monomer is a neurotoxin.

TEMED:	N,N,N',N' Tetra methylethylene diamine	20.0 ml	4.0% V/V
	Acetic Acid (glacial)	216.0 ml	43.2%
	Water to 500 ml		
Acrylamide:	N,N Methylene bis acrylamide	4.0 gm	0.4%
	Acrylamide monomer	615.0 gm	61.5%
	Water to 1 liter		

0.9 N acetic acid: 104 ml concentrated acetic acid /2000ml with distilled water

Stain:	Napthol Blue Black (amido schwarz)	2.0 gm	0.1%
	Mix first before adding the NBB to it:		
	Ethanol (200 proof)	600.0 ml	30.0%
	Acetic acid	104.0 ml	0.9%
	Water to final 2 liters		

1) Place 8 ml of acrylamide stock solution in a 125 ml vacuum flask. Connect the rubber/tygon tube vacuum line up to the side port of the flask, and use a rubber stopper that is much too large to fit into the flask so it sits on top of the flask in order to make it easy to break the vacuum seal when needed. Turn on the vacuum and allow the air to be removed from the solution (you will see bubbles coming out of the solution, rapidly at first and slowing down after 10-20 seconds). When the bubbling slows down, do not turn off the vacuum first as the vacuum in the flask may pull oil or other garbage from the vacuum line back into the flask. Break the vacuum by removing the rubber stopper first then turn off the vacuum. Do not leave the flask hooked up to the vacuum for more than a minute as it will evaporate the liquid and reduce the volume significantly. The goal is to just remove the extra air from the solution to minimize the formation of bubbles when the gel polymerizes.
2) In another 125 ml vacuum flask place:
    a. 4.8 gm urea
    b. 30 mg ammonium persulfate
    c. 20 ml distilled water
    d. 4 ml TEMED stock solution with 50 µl 100 % TEMED (add this to the 4ml of TEMED stock solution; this is required to achieve gel polymerization within a reasonable time.)
    e. Mix well and remove the air from this solution on the vacuum line in the same manner as in 1.
3) Set up gel cassettes (Jule Snap-a-gel from Colonial Scientific, VWR or ThermoFisher, 1.5 mm empty gel cassettes, 10 well comb). Test the comb for fit (some are too tight), mark the bottom of the well and number wells if desired, and put tape on the bottom of the cassette (if they come without it) to keep the gel from running out the bottom. Place cassette in a test tube rack (the kind with pegs) to support cassette.
4) Add the urea mixture to the acrylamide and swirl gently to mix. The recipe makes ~ 32 ml total.
5) Pipet in gel mixture to just below the top of the cassette and if necessary gently tap to remove any bubbles.
6) Inset the comb checking that there are no bubbles at the bottom of the wells and with a

pasture pipette very slowly and gently add 0.9 N acetic acid around the comb to remove air at the top and set up a gel-acetic acid interface at the top of the gel.

7) Let stand for 1.5-2 hours at room temperature – until wells have clearly formed.
8) Remove tape from cassette. Place cassettes in the electrophoresis apparatus with combs facing the inside of the chamber.
9) Add 0.9 N Acetic acid to both the inner and outer chambers (outer chamber about half full).
10) Remove the combs slowly and aspirate the wells with the acetic acid in the chamber so you get rid of salts and see a clear gel interface.
11) Hook up the electrodes to the power supply **reversing the electrodes from the orientation used for SDS gels.** For these gels the positive electrode has to be at the top of the gel and the negative at the bottom, the opposite the way SDS gels are run. In this system the protamines and histones are positively charged and need to move away from the top of the gel and down into it.
12) Pre-electrophorese the gel at 130V for 3 hours. This is running the gel without anything layered on top of the wells. If you cannot set the power supply to a constant voltage then monitor the current running through the gel and as the current flowing through it drops over time you should adjust the voltage so it stays close to 130V. The higher the voltage and current flow through the gel the more heating you will get inside the gel, which will result in “not so pretty” gel bands.
13) Turn off the power and remove the top.
14) Aspirate the wells gently with the acetic acid above the gel top so you can see the gel tops.
15) Carefully add your protein sample (dissolved in final 20% Sucrose, 0.9N Acetic acid, 0.5M β-mercaptoethanol using a loading pipette so it layers properly on top of the well) to each well - 10ug total protamine or protein is a good amount.
16) Hook up **(making sure the positive and negative electrodes are reversed with respect to how they are used for SDS gels; so the positive electrode is at the top of the gel)** and turn power on
17) Electrophorese at a constant voltage setting of 130V for approximately 1 hr 15 min to resolve the protamines (you may need to run a test gel with protamine to determine the needed time for your cassette and power supply.
18) Turn off power and remove the gels by breaking open the cassette with the cassette opener, mark the upper 1st well by cutting a hole with the end of a 1ml pipet 1 hole =A, 2 holes=B.
19) Stain the gel in a large flat container that can be closed (large petri dish may work if gel is the typical small one) with enough Napthol blue black stain to completely cover the gel - 1 gel per container (Coomassie Blue stain can be used as an alternative stain).
20) Stain overnight.
21) Pour off stain and place the gel in the destainer (filled with distilled water with a squirt of 0.9 N acetic acid added)
22) Place the top on the destainer and turn the power supply on.
23) Destain until the background is clear, changing water as needed, turn power off and place the gels in a tupperware box fill with 0.9N acetic acid
24) The gels can also be destained by diffusion by placing the gel in a container with a large volume of 0.9N acetic acid (or water with a small amount of the acetic acid added to it) and letting the stain diffuse out of the gel. If this method is used, the solution will need to be changed 3-4 times over the course of 24-48 hr.