SDS-PAGE

Stock solutions:

SDS-PAGE itself

Acrylamide: 30% acrylamide, 0.8% bis-acrylamide. Store at 4 C in the dark.

APS: 25 mg of ammonium persulfate in 100 ml of H_2O. Store at 4 C.

8.8 buffer: 1.5 M tris pH=8.8, 0.4% SDS

6.8 buffer: 0.5 M tris pH=6.8, 0.4% SDS

ButOH/H_2O: Mix 1-buthanol and H_2O and leave it for a while. You will obtain two phases, the upper one is buthanol saturated with H_2O and is what you will use.

TD 4x - ßSOH: 0.25 M tris pH=6.8, 8% SDS, 35% glycerol and an small quantity of bromophenol blue

TD 4x + ßSOH: 0.25 M tris pH=6.8, 8% SDS, 35% glycerol, 2.5% 2mercaptoethanol and an small quantity of bromophenol blue

Reservoirs buffer x 10: There is an stock of Tris/glycine buffer. Mix 1 l of that stock with 50 ml of 20% SDS.

SDS-PAGE staining:

Fixing solution: 20% trichloroacetic acid.

Staining solution: Disolve 0.25 g of Coomasie brilliant blue in 45 ml of methanol. Add 45 ml of H_2O and 10 ml of acetic acid.

Destaining solution: There is a common stock in the lab.

SDS-PAGE Transfer:

SDS-PAGE transfer stock: 1 M bicine pH=9 (NaOH)

Blot staining solution: 0.1% Poinceau red, 1% acetic acid.

PT: 20 mM NaPi pH=7.5, 150 mM NaCl, 0.1% Tween 20

Blocking solution: 3% BSA in PT

Developer: An small quantity of 3,3'-diaminobenzidine in 10 ml of 50 mM Tris pH=7.4 and 2.5 µl of H_2O_2

Preparation of the SDS-PAGE gels:

Using gloves, wash the glass plates (a big one and a small one for each gel) with ethanol.

Mount a sandwich with the glass plates and two spacers.

Introduce the sandwich in the support piece, putting the big glass plate in contact with the acrylic bloke.

Put this setup on a flat surface and adjust the upper screws.

Check that the lower borders of the glass plates and of the spacers are well aligned and adjust the lower screws.

Fix this setup agains the rubber piece.

Prepare two solutions, one of them will be the separating gel and the other the stacking gel:

For **one 0.5 mm thick gel of 13% acrylamide**:

Separating gel	Stacking gel
9 µl APS	5 µl APS
712 µl H_2O	746 µl H_2O
577 µl 8.8 buffer	
	300 µl 6.8 buffer
1000 µl acrylamide	140 µl acrylamide

For **two 0.5 mm thick gels of 13% acrylamide**:

Separating gel	Stacking gel
20 µl APS	10 µl APS
2 x 771 µl H_2O	2 x 746 µl H_2O
2 x 625 µl 8.8 buffer	
	600 µl 6.8 buffer
3 x 722 µl acrylamide	288 µl acrylamide

Quickly, add the TEMED (same volume as APS) to the separating gel, invert the tube two or three times and pour 3 x 700 µl in each sandwich. Cover the poured gel or gels with butOH/H_2O.
Using gloves, clean the comb or combs with ethanol and check that the separating gels have already polymerized. If that is the case, decant the buthanol and wash each sandwich several times with H_2O.
Add the TEMED (same volume as APS) to the stacking gel, invert the tube two or three times and pour about 700 µl of the gel in each sandwich. Insert the combs and refill well with the remaining stacking gel.

Preparation of the samples:
Dilute each sample with TD x4 until it is x1 and heat it at 90 C for 5 min. The colour of the sample must be blue. If it changes to yellow add an small quantity of concentrated acid and if it changes to translucid add an small quantity of concentrated alcali.

SDS-PAGE:
Prepare 300 ml of Reservoirs buffer, diluted 1x.
Check that the stacking gel is already polymerized and remove the combs. Remove the excess of polymerized acrylamide with a paper. If you are using 0.5 mm thick gels, is a good idea check that the holes formed by the combs are free of acrylamide by using a fine needle.
Mount the two support pieces of the gel sandwiches against the U-shape rubber piece. If you are using only one gel, mount an small glass plate in the other support piece. This new setup will form the upper reservoir. Put it on the cubette. Fill the upper reservoir with Reservoirs buffer.

Pour the samples in the preformed holes. If you have 10 holes for gel you can put 15 µl in each hole. If you have 15 holes for gel you can put 6 µl in each hole.
Pour the remaining of the Reservoirs buffer on the cubette and run the electrophoresis. If you are using 0.5 mm gels, you can apply 70 mA for each gel.
The SDS-PAGE is finished one minute or so after the blue line has run out of the gel. Dismount the whole setup and discard the stacking gels.

Staining of a SDS-PAGE:
Incubate the gels in fixing solution for 5 min at 55 C with shaking.
Discard de fixing solution and incubate the gels in staining solution for 10 min at 55 C with shaking.
Recycle the staining solution. Wash the gels with water and incubate them in destaining solution for 10-15 min at 55 C with shaking.
The gels are stored in H_2O with acetic acid until they are dried.
Dry the gels between two cellophan sheets. Use the gel drier at 80 C for 30 min.

Western blot:
Prepare transfer solution by mixing 980 ml of H_2O with 110 ml of methanol and 11 ml of SDS-PAGE Transfer stock.
Incubate the gels in about 100 ml of transfer solution for 15 min with shaking. In the mean time, incubate one piece of PVDF for each gel in methanol for 1 min. After that, pour the methanol and replace it with transfer solution.
Prepare each transfer sandwich by putting...
- the black piece of the transfer sandwich
- 1 wetted white pad
- 1 wetted3MM paper sheet
- transfer solution
- 1 gel
- transfer solution
- 1 blot of PVDF
- Roll a pipet on the setup, in order to remove potential bubbles.
- transfer solution
- 1 wetted 3MM paper sheet
- 1 wetted white pad
- Close the sandwich with the white piece of the transfer sanwich

Mount the sandwiches in the transfer device, putting their black sides near the black side of the transfer device.
Fill the cubette with the transfer solution and transfer at 100 V for 30 min, with magnetic stirring.

Remove the PVDF blots, wash them briefly with water and incubate them in Blott staining solution for 1 min with shaking Wash with water and photocopy the blots,
Incubate the blots in blocking solution for 1 hour with shaking.
Add the antibody to the blocking solution and incubate the blots for 30 min with shaking.
Wash the blots 3 times by incubating 5 min in PT with shaking.
Add the secondary antibody in blocking solution and incubate the blots for 30 min with shaking.
Wash the blots 3 times by incubating 5 min in PT with shaking.
Incubate in developing solution. When the bands are intense enough, wash with water and dry the blots.