Preparation of SDS gels:

Materials Needed:
40% Polyacrylamide
1M Tris Separating
1M Tris Stacking
10 % Ammonium Persulfate
10% SDS
TEMED
Water
Isopropanol
15 mL Falcon Tubes
1.5 mL Eppendorf Tubes
SDS plates
SDS well combs
Electrophoresis Chamber
Pipets

Protocol:

1) Components from tables 1 or 2 are combined in a15mL Falcon tubes adding the 10% Ammonium Persulfate last and none of the TEMED[1].
2) Take a 1mL aliquot from the falcon tube with the separating layer components and transfer it into a 1.5mL Eppendorf tube.
3) Add 4 µL of TEMED, invert the Eppendorf tube and put 0.5 mL between the casting plates of the SDS gel.
4) On top of the separating layer, add 1mL of isopropanol and allow gel to solidify for 5 minutes.
5) Remove the Isopropanol layer from the top of the gel with a kimwipe and let dry for a few minutes to ensure all the Isopropanol has evaporated.
6) Add 8 µL of TEMED into the remaining separating solution and invert.
7) Transfer approximately 3mL of the separating solution between the casting plates, on top of the 0.5mL separating layer.
8) Then on top of the separating layer add Isopropanol until the casting plates are over-flowing.
9) When gel has solidified, remove the isopropanol layer and let dry for a few minutes.
10) Add 10 µL of TEMED into the stacking solution and invert.
11) Add the stacking solution on top of the separating solution until the casting plates are overflowing and place the casting comb in-between the plates.
12) When the stacking gel has solidified the comb is removed.
13) Load 10 µL of the samples made [2].
14) Run the gel at 60V until the loaded samples just pass the separating-stacking interface, then increase
15) Increase the voltage to 160V and let the samples run until the samples reach approximately two centimeters from the bottom.

16) Increase voltage to 200V and let the samples run off the bottom of the gel.

Table 1: To run SDS of CCH2.

	Stacking	Separating
Gel %	10	10
40% Polyacrylamide (ml)	0.5	2.5
1M TRIS (ml)	0.5	2.5
10 % Ammonium Persulfate (mL)	0.04	0.1
10% SDS (ml)	0.04	0.1
TEMED (ml)	0.004	0.004
Water (ml)	2.92	4.8
Total Volume (ml)	4	10

Table 2: To run SDS of NIC C, D, E, F and X.

	Stacking	Separating
Gel %	14	14
40% Polyacrylamide (ml)	0.625	3.5
1M TRIS (ml)	0.625	2.5
10 % Ammonium Persulfate (mL)	0.05	0.1
10% SDS(ml)	0.05	0.1
TEMED (ml)	0.005	0.004
Water (ml)	3.65	3.8
Total Volume (ml)	5	10

Reference:

[1] Chang Bioscience 40% polyacrylamide SDS page calculator.

Other Protocols:

[2] Preparation of samples to run SDS of CCH2, NIC C, D, E, F and X: