iGEM 2016 – SDU

Title: SDS-page	**Date issued:** 2016.07.14
SOP number: SOP0024_v01	**Review date:** 2016.07.14
Version number: 01	**Written by:** Brian Kenn Baltzar

1. Purpose

To cast polyacrylamide gel and run a SDS Gel electrophoresis

2. Area of application

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
SDS - casting Apparatus			
SDS - running Apparatus			
pH-meter			
Steril filter			
Syringe			
Needle			

4. Materials and reagents – their shelf life and risk labelling

Name	Components (Concentrations)	Manufacturer / Cat. #	Room	Safety considerations
Blue pipette tips		Contact Lab-manager	Micro storage	
Tris-base			Micro Chem	
dH_2O				

Acrylamide	SDS-Gel room
bis-methylen e-acrylamide	SDS-Gel room
Glycerol	Micro Chem
HCl	Micro Chem
Ammonium persulfate	Micro Chem
SDS (Sodium dodecyl sulfate) (Natriumlaurylsulfat)	Micro Chem
TEMED (Tetramethylethylenediamine)	SDS-Gel room

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

JMJ_SOP0001 ON culture of E.coli

7. Environmental conditions required

8. Procedure

1. Wash all of the hardware - make sure they are clean otherwise it may leak.
2. Assemble the glass plates and spacers and fix them in the plastic mounting devices.
3. Mount the assembled glassplates on the casting stand and make sure the stand is level.
4. With a marker, place a mark on the glass plate 1 cm below the teeth of the comb. This will be the level to which the separating gel is poured. Remove the comb.

Separation gel:

4. Add the APS to the Separation gel-mix.
5. Mix by gently inverting 5 times
6. Add the TEMED - Cast the gel immediately after addition of TEMED up to the point marked at step 4.
7. Carefully add H_2O up to the edge of the glassplates.
8. When the remaining gel-mix in the falcon tube has polymerized, remove the H_2O from the top of the gel by tilting the whole assembly and using a napkin to remove the excess H_2O.

Stacking gel:

9. Add the APS to the Stacking gel-mix.
10. Mix by gently inverting 5 times
11. Add the TEMED - Cast the gel immediately after addition of TEMED up to the top.
12. Insert the comb onto the top of the gel.
13. Carefully remove the comp when the remaining Stacking gel-mix in the falcon tube has polymerized.

Gel electrophoresis:

14. Fix the gel on the gel mounting device and add 1x TGS running buffer to the reservoir.
15. Add 20µL 2x SDS-loading buffer to the prepared cell dilutions.
16. Boil the cells in a thermo mixer at 95ºC for 5 minutes.
17. Place the samples on ice for 1 min.
18. Spin briefly and leave at room temp.
19. Load 5µL of a protein marker in the 2^{nd} well of the gel.
20. Load 20-30 µL of each sample in the following wells.
21. Run the gel at 200 V
22. Stop the electrophoresis when the bottom protein band is about 2-4 cm. above the bottom.
23. Disassembly the glass plates and continue with staining.

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
ON Culture		Liquid bacterial waste	
Once use plastic		GMO yellow waste	

10. Time consumption

- Total-time 5-6h.
- Hands-on-time 60 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.07.14 / BKB	01	The SOP has been written
16.10.14/	01	The SOP has been approved

12. Appendixes

Buffers and solutions:

20% Separation gel-mix (Bio-rad recipe) 15 mL

10 mL	30% Acrylamide/bis	
3.75 mL	1.5M Tris-HCl, pH 8.8	
150 µL	10% SDS	
1.03 mL	diH2O	
7.5 µL	TEMED	ADD JUST BEFORE CASTING!
75 µL	10% APS	ADD JUST BEFORE CASTING!

4% Stacking gel-mix (Bio-rad recipe) 15 mL

1.98 mL	30% Acrylamide/bis	
3.78 mL	0.5M Tris-HCl, pH6.8	
150 µL	10% SDS	
9 mL	diH2O	
15 µL	TEMED	ADD JUST BEFORE CASTING!
75 µL	10% APS	ADD JUST BEFORE CASTING!

X% Separation gel-mix (Bio-rad recipe) 15 mL

0.5 * X mL	30% Acrylamide/bis	
3.75 mL	1.5M Tris-HCl, pH 8.8	
150 µL	10% SDS	
11.03-(0.5*X) mL		diH2O
7.5 µL	TEMED	ADD JUST BEFORE CASTING!
75 µL	10% APS	ADD JUST BEFORE CASTING!

2x SDS-PAGE Buffer

3.75 mL	Tris-HCl pH 6.8, 0.5M
24.0 mL	Glycerol
300 µL	1.0% Bromophenol blue eller xylene cyanol (MEGET Lidt)
6.0 mL	10% SDS
-> 30 mL	diH_2O

Acrylamide/Bis 30%

87.60 g	Acrylamide (29.2g / 100mL)
2.40 g	N'N'-bis-methylene-acrylamide
-> 300 mL	diH_2O

Filter and store at 4ºC

1.5 M Tris-HCl, pH 8.8 (150 ml)

27.23 g	Tris-base (18.15 g/100 ml)
80 mL	diH2O
Adjust to pH 8.8 with 6N HCl	
-> 150 mL	diH2O

Store at 4ºC

0.5 M Tris-HCl, pH 6.8

6 g	Tris-base
60 mL	diH2O

Adjust to pH 6.87 with 6N HCl
-> 100 mL diH2O
Store at 4ºC

10% APS
0.10 g Ammonium persulfate
1 mL diH2O

10% SDS
10.00 g SDS
60 mL diH2O
Dissolve with gentle stirring
-> 100 mL diH2O