JUL 10, 2019

14 SDS-PAGE

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GUIDELINES

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.49qgz5w

Protocol Citation: Tjusls China 2019. 14 SDS-PAGE. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.49qgz5w>

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Protocol status: Working
We use this protocol and it's working

Created: Jul 08, 2019

Last Modified: Jul 10, 2019

PROTOCOL integer ID:
25616

SDS-PAGE Separation of gel samples	Scope of application
6 % sample gel	50-150kD
8 % sample gel	30-90kD
10 % sample gel	20-80kD
12 % sample gel	12-60kD
15 % sample gel	10-40kD

MATERIALS

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- ☒ TEMED Bio-rad Laboratories Catalog #1610801
- ☒ 40g Acrylamide/ Bisacrylamide (29:1); Premixed Powder G-Biosciences Catalog #786-506
- ☒ Coomassie brilliant blue G-250 Bio Basic Inc. Catalog #CB0038.SIZE.100g
- ☒ SDS, 10%(w/v) solution Bio Basic Inc. Catalog #SD8118.SIZE.100ml
- ☒ Tris pH 6.8 Contributed by users
- ☒ 10 % Ammonium persulfate Contributed by users Catalog #/
- ☒ 1M Tris(PH=8.8) Contributed by users Catalog #/

SAFETY WARNINGS

Be careful of 30 % Acr-Bis M and TEMED owing to their potential harm.

BEFORE START INSTRUCTIONS

In general, 15 % sample gel is used, so here is only the composition of 15 % sample gel.

Composition of the 15 % separated of the gel sample(4mL):

Composition	volume (mL)
Distilled water	0.4
30 % Acr-Bis (29 : 1)	2
1M Tris pH=8.8	1.5
10 % SDS	0.04
10 % Ammonium persulfate	0.04
TEMED	0.002

Composition of concentrated sample gel(1mL):

Composition	volume (mL)
Distilled water	0.7
30 % Acr-Bis (29 : 1)	0.165
1M Tris pH=6.8	0.125
10 % SDS	0.01
10 % Ammonium persulfate	0.01
TEMED	0.001

- 1 Mount the gels in the vertical electrophoresis apparatus.
- 2 Load samples under the cathode buffer. Apply 10 μ L sample volumes to 0.7 $\times$ 5 mm sample wells.

10 µL

- 3 Set running conditions appropriate to your type of gel. We usually set 200V to run at the beginning, after 10 minutes change the voltage into 300V for another 30 minutes.
- 4 Protein can be visualized directly in the gel by Coomassie staining or silver staining. We usually choose Coomassie staining, then transfer the gel to the decolorizing solution and remove the dye solution.
- 5 Recording with gel imager.