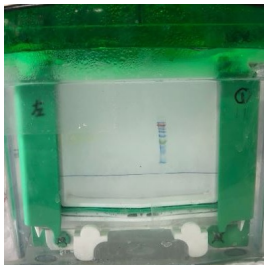
Jul 10, 2019

14 SDS-PAGE



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

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Guidelines

	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

MATERIALS

- 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
- 10 % Ammonium persulfate **Catalog #**/
- 1M Tris(PH=8.8) **Catalog #**/

Troubleshooting

Safety warnings

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

Before start

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 10μL sample volumes to 0.7 × 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.