Electro-elution of proteins from SDS polyacrylamide gels

This is a description of electro-elution of proteins from polyacrylamide gels using the Bio-Rad Model 422 Electro-Eluter apparatus. Six samples can be electroeluted simultaneously. Wear gloves throughout the procedure to prevent contamination of the samples.

1. Gel electrophoresis and protein visualization

- Load sufficient amount of protein on a preparative SDS polyacrylamide gel with a reference well giving an optimum separation of the protein to be eluted.
- After electrophoresis briefly rinse the gel in H_2O. Incubate the gel for 5 min in 20ml Copper Stain on a shaker. Destain the gel 3min in 100ml H_2O. The gel can be stored in H_2O for several days without severe loss of staining or resolution.
- Place the gel on a glass plate on black paper. Protein bands appear dark against a semi-opaque whitish-blue background. With a clean scalpel cut a gel slice containing the desired protein band.
- Wash the gel slice 3 x 5min with 5ml Copper Destain and 1 x 5min with 5ml Elution Buffer in a 15ml Falcon tube.
- Place the gel slice on a piece of Parafilm and cut it into smaller pieces to fit in the electro-eluter glass tubes.

2. Protein elution

- Soak new **membrane caps** (clear: MWCO 12-15 kDa, green: MWCO 3.5 kDa) in Elution Buffer for at least 60min at 60°C. Reused membrane caps can be used without any pretreatment.
- Place a **frit** in a **glass tube** so that it is flush with the bottom of the frosted end. Place the glass tube with the frit in the electro-eluter module so that the open end is even with the top of the grommet.
- Place the pre-wetted **membrane cap** in the bottom of the **silicon adaptor**. Fill the adaptor with Elution Buffer and remove air bubbles around the dialysis membrane by pipetting up and down.
- Slide the **silicon adaptor with the membrane cap** onto the bottom of the **glass tube with the frit**. Air bubbles are expelled from the frit by pulling the silicon adaptor partially on and off a few times.
- Fill the tube with Elution Buffer almost to the top and place the **pieces of the gel slice** in the tube.
- Place the electro-eluter module in the buffer chamber. Fill the lower buffer chamber with ca. 600ml of Elution Buffer and the upper chamber with ca. 100ml. Remove air bubbles from the bottom of the membrane caps with a Pasteur pipette. Place a stirbar in the lower tank.
- Elute at 10mA per glass tube constant current for 3 to 5 hours with vigorous stirring. Increase elution time when using high percentage gels or eluting high molecular weight proteins.
- SDS present in the Elution Buffer concentrates in the lower buffer chamber during the run. SDS can efficiently be electrodialyzed from the collected sample. Exchange the lower buffer at the end of the run for fresh Elution Buffer without SDS and continue elution for 30-60 minutes.
- After elution is completed, remove the electro-eluter module from the tank and drain the upper buffer chamber. Pull out the first glass tube, remove the buffer above the frit with a pipette, and then remove the silicon adaptor together with the membrane cap from the bottom of the glass tube.
- Pipet the solution in the membrane cap (400-500µl) into a microfuge tube. Carefully rinse the membrane cap with 200µl fresh Elution Buffer, combine with the other solution. Repeat for the other tubes. Spin eluted protein solutions 2min at 10 000xg.
- The membrane caps can be reused several times when carefully washed in Elution Buffer and stored in Elution Buffer containing 0.05% NaN_3 at 4°C.

3. Reagents

• 10 x Elution Buffer:	250mM Tris(hydroxymethyl)aminomethane	30g
	1920mM Glycine	144g
	1% SDS	10g
	H_2O	to 1000ml
	pH 8.3 (unadjusted), store at RT	
• Elution Buffer:	25mM Tris(hydroxymethyl)aminomethane	3.0g
	192mM Glycine	14.4g
	0.1% SDS	1.0g
	H_2O	to 1000ml
	pH 8.3 (unadjusted), store at RT	
• Elution Buffer - SDS:	25mM Tris(hydroxymethyl)aminomethane	3.0g
	192mM Glycine	14.4g
	H_2O	to 1000ml

	pH 8.3 (unadjusted), store at RT	
• Copper Stain:	300mM $CuCl_2$.2 H_2O	10.2g
	H_2O	to 200ml
	(can be reused several times)	
• Copper Destain:	250mM Tris(hydroxymethyl)aminomethane	6.06g
	250mM EDTA-Na_2	18.62g
	H_2O	to 200ml
	pH 9.0 (NaOH)	

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