Blue-Native PAGE

(ref Shagger, H., and Von Jagow, G., Analytical Biochemistry, 1991, 199, 223-231)

Solutions:

3xGB - 3xGel buffer (1.5M aminocaproic acid, 150 mM Bis-tris, pH 7.0)
19.68g Aminocaproic acid
3.14g Bis-tris
ad 100 mL H20

Cathode buffer (15mM Bis-tris, 50 mM Tricine, pH 7.0)
3.14g Bis-tris
8.96g Tricine
ad 1000mL H20

Blue cathode buffer (Cathode buffer cont. 0.02% Serva Blue G (SBG)
100 mL Cathode buffer
0.02g SBG

Anode buffer (50mM Bis-tris, pH7.0)
20.93 g Bis-tris
ad 2000 mL H20

AB - Acrylamide/bisacrylamide mix (48% acrylamide, 1.5% bisacrylamide (99.5T, C))
24.0 g acrylamide
0.75 g bisacrylamide
ad 50 mL H20

MB2 - 0.5 ml 3xGB, 0.5 ml 2M Aminocaproic acid, 4µl 500mM EDTA

SBG - sample buffer (750 mM Aminocaproic acid, 5% SBG)
3.75 mL 2M Aminocaproic acid
0.5g SBG
ad 10 mL H20

AC - 2M aminocaproic acid (13.12g/50 mL)

LM - 10% Lauroyl maltoside (0.1g/mL)

AP - 10% Amonium persulfate (0.1g/mL)

	Stacking 4% gel		Separating 6% gel		Separating 15% gel	
	2.5 ml	5 ml	5 ml	10 ml	5 ml	10 ml
3xGB	0.82 ml	1.64ml	1.65 ml	3.3 ml	1.65 ml	3.3 ml
AB	0.2 ml	0.4ml	0.6 ml	1.2 ml	1.5 ml	3.0 ml
water	1.45 ml	2.87 ml	2.72 ml	5.44 ml	0.84 ml	1.68 ml
glycerol	0	0	0	0	1 ml	2 ml
AP	30 µl	60 µl	30 µl	60 µl	5 µl	10 µl
TEMED	3 µl	6 µl	2 µl	4 µl	2 µl	2 µl

Preparation of samples

Use isolated mitochondria or 15 000xg sediments of postnuclear supernatants, or digitonin treated fibloblasts. Perform all steps at 0-4°C:

- resuspend mitochondria in MB2 at 1-3 mg protein/ml
- add LM to final concentration 1%, mix well and leave 15 min
- centrifuge 20 min at 20 000xg
- add SBG to supernatant to final concentration of 0.25%
- apply samples to gel

Preparation of gel and electrophoresis

Use Miniprotean Bio Rad apparatus with 1 mm spacers and combs:

- mount together the glass "sandwich" cassettes
- prepare 5 ml of 6% and 15% separating gel mixture according to table
- fill the tubing and about 1 cm of casette height with water
- fill the front reservoir of gradient mixer with 2.8 mL 6% mixture and the rear reservoir with 2.3 mL of 15% gel mixture, put the conical insert into rear reservoir
- fill the gel cassette by means of underlaying the gel under water using peristaltic pump at speed 9
- after polymerization wash gel surface with 1x GB
- prepare 2.5 ml of stacking gel mixture according to table, insert comb and fill up to the top with stacking gel mixture
- after polymerization remove comb and wash wells with 1x GB
- fill the spaces in wells with blue cathode buffer and underlay the samples into wells (sample volume should not exceed 20 µl/well (10 well comb), the amount of protein should be between 10-30µg prot.)
- place gel cassettes into apparatus and fill upper and lower reservoir with blue cathode and anode buffer
- run 15 min at 40V, then at 80V until the dye reaches 2/3 of the gel, than replace blue cathode with cathode buffer and continue electrophoresis until the dye front reaches the end
- remove gel from between the plates
- use for Western blotting or stain proteins in gel (30 min) and destain the background (1-2h, 2x change of the destain solution)
- for second dimension electrophoresis, cut the gel slice with separated proteins and use directly or freeze and store at - 20°C