UNIVERSITY
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Biological and Chemical
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GoRNA
STRUCTURAL BIOLOGY GROUP

Whatman® Elutrap electroelution system

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Whatman® Elutrap electroelution system (I)

The Elutrap System is designed to isolate nucleic acids and proteins from agarose or polyacrylamide gel slices by electroelution. Samples are purified with excellent recovery into volumes as low as 200 µL, without requiring sample pretreatment or special buffers.
The Elutrap System can be used with most horizontal gel electrophoresis chambers. The Elutrap Electrophoresis Chamber allows for the most efficient flow of current through the device and can be used for up to four samples simultaneously.

Whatman® Elutrap electroelution system (II)

Gel slices are placed in the middle of the Elutrap device, which is then placed into a horizontal electrophoresis chamber. Molecules migrate from the gel slice into a trap area formed by BT1 and BT2 membranes. The membrane placement is adjustable, allowing final elution trap volumes to be optimized for the particular assay. The Elutrap System can also be used for the concentration of dilute solutions. Versatile system can be used for nucleic acids and proteins. Purifies nucleic acids 14 bp to 150 Kb; proteins larger than 3 to 5 kD. No special buffers is required for elution.

Purification of RNA using Elutrap

1. RNA synthesis

Prepare 3-5 reactions (depending on efficiency of transcription):

Component	Volume for one reaction
DNA template	3000 ng
IVT buffer (10x)	20 µl
DTT	20 µl
A/U/C/G	10 µl each (40 µl total)
RNase out	2.5 µl
RNAP T7	2.0 µl
DMSO	variable
water	To 200 µl

(10x IVT reaction buffer: 400 mM Tris (pH 7.9-8.0), 250 mM $MgCl_2$, 20mM spermidine)

Run reactions for 5h in 37°C.

Add 10 µl of DNase I to each reaction and incubate for 30 min in 37°C.

Add 20 µl of 500 mM EDTA (RNase free), votex and incubate 1 min in RT to dissolve the pellet.

Purification of RNA using Elutrap

2. **Electrophoresis**

- Prepare polyacrylamide gel in urea buffer:
- Wipe 40 cm long plates, spacer and 1 well comb with ethanol
- Assemble plates and spacers
- Prepare gel of appropriate percentage (for 100-200 nt, 4-6% gel)
- After pouring the gel insert the comb and let it polymerize for 1h
- When the gel polymerizes, prepare the sample: mix RNA with loading dye (use 600 µl of loading dye for 400 µl of RNA sample), boil for 5 min and cool down on ice.
- Remove the comb and put the gel to electrophoretic tank and pour 1x TBE buffer. Wash the well using a sterile syringe (use fresh syringe each time). Load the sample using long tips and run the gel in 400-500V for 5-6h.

Purification of RNA using Elutrap

3. **Preparing and loading Elutrap (I)**

- Spray Elutrap tank with RNase Zap and rinse generously with MQ water. Fill the tank with 1l of 1x TBE buffer and leave in a cold room (4°C) to cool down.
- Prepare the bench: place a long piece (ca. 50x60 cm) of plastic foil on a bench (make sure that the foil does not touch anything before laying it down). Sprinkle the foil with MQ water.
- Transfer the gel from the plate on the foil and wrap it. Place a white sheet of paper under the gel.
- Using hand UV lamp (short wave length) identify the band corresponding to RNA (it should appear as a shadow on the gel). Try to limit time of UV exposure since it could damage the RNA.
- Excise the band from gel with sterile razor, cut it into smaller pieces, transfer to 15 ml falcon tube and place the tube in ice
- Place a layer of paper towel on the bench (touch the towel only on its' borders)
- Prepare two Elutrap chambers for each RNA: spray the chambers, tweezers and Elutrap screwdriver with RNase Zap, rinse with MQ water and place on a paper towel

Purification of RNA using Elutrap

3. **Preparing and loading Elutrap (II)**

- Prepare Elutrap membranes: two transparent membranes (in package with black circle) and one white membrane (package with blue circle) for each chamber
- Spray your hands with RNase Zap
- Unscrew the chambers
- Use the tweezers to place transparent membranes at both ends of each chamber
- Move the blocks at one end of the chamber (move two blocks to the transparent membrane) to create space and place the white membrane
- Screw the chamber tightly
- Remove the falcon with gel slices from ice, wipe on paper towel and distribute gel slices into two chambers
- Using RNase-free pipette tip, fragment the gel into tiny pieces
- Place the chambers into Elutrap tank: one membrane on right side (black cable), two membranes on the left (red cable), make sure that the hole on the left side of the chamber is open
- Fill the chambers with 1x TBE until the level of the buffer is 1 mm below the boarder of chamber
- Pour 1x TBE to the tank, until it reaches the same level as inside of the chambers
- Run reaction in 100V overnight

Purification of RNA using Elutrap

4. **Processing of the samples**

! Collect the RNA form between the membranes (use long tips and pipette the solution up and down a few times to remove RNA collected at the transparent membrane, be careful not to touch the white one since it breaks easily)

! Concentrate the RNA on Vivaspin columns (choose cut-off depending on the size of RNA) until volume of the sample is ca. 200 µl

! Purify the RNA using spin columns (for RNA shorter than column's cut-off, double the volume of ethanol), use 2 columns per one sample to avoid overloading

! Elute columns with RNase-free water

! Measure the concentration on Nanodrop

! Run the reactions on polyacrylamide gel in urea buffer to verify RNA integrity

Thank you