Macaloïd/Roche method

Protocols

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1. Introduction

- This is a combination of the macaloïd method and the Roche RNA isolation kit, for isolation of total RNA for microarray analysis.

2. Macaloïd part of the protocol.

1. Grow cells till wanted OD600.
2. Collect 10-20 ml culture, 1 min at 10.000 rpm (RT).

   Use 10-15 OD equivalents : OD 0.1 → 100ml
   OD 1.0 → 10ml

3. Discard as much medium as possible and immediately freeze in liquid nitrogen.

   If there is enough time :resuspend pellet quickly in 400 µl TE (DEPC), pipet it into a 2ml screw cup and freeze tube in liquid nitrogen.

4. Store pellet at -80°C.

5. Thaw cell pellet on ice.
6. Resuspend pellet in 400 µl TE (DEPC) if not done yet.
7. Add to screw-cap tubes containing (should be prepared in advance):

   0.5 g glass beads
   50 µl 10% SDS
   500 µl phenol / chloroform:IAA

   Premix 300 µl chloroform:IAA (24:1) and 300 µl phenol (acid phenol, RNA grade, Sigma P4682) and take 500 µl of the organic phase.

   Macaloïd layer (150-175 µl – not exact as it is very viscous)
8. Place in **bead beater**. 2x pulses ('homogenize'), 1 min interval on ice.
   - *L.lactis* 2x 45"
   - *S.pneu* and *B.sub* 2x 60"
9. Centrifuge **10** min at 10.000 rpm (4°C).
10. Transfer upper phase to fresh tube, add 500 µl chloroform:IAA (24:1)
11. Centrifuge **5** min at 10.000 rpm (4°C).

3. Roche part of the protocol.

12. Transfer 500 µl of upper phase to fresh tube, add 2 volumes (1 ml) of lysis/binding buffer (● green cap) and mix by pipetting up and down.
13. Combine filter and collection tubes and pipette 800 µl to upper reservoir.
14. Centrifuge 15 sec at 8600 rpm.
15. Discard flow through and pipette remainder of sample (~700 µl) to upper reservoir.
16. Centrifuge 15 sec at 8600 rpm and discard flow through.
17. Add 100 µl DNaseI mix. (90 µl DNase buffer and 10µl DNaseI).
18. Incubate 20-30 min at 15-25 ºC.
19. Add 500 µl wash buffer I (● black cap) to upper reservoir, centrifuge as before and discard flow through.
20. Add 500 µl wash buffer II (● blue cap) and centrifuge as before.
21. Add 200 µl wash buffer II (● blue cap) and centrifuge 2 min at max speed.
22. Discard collection tube and insert filter tube in a sterile 1.5 ml eppendorf tube.
23. Add 50 µl elution buffer (o colorless cap) directly on filter in upper reservoir and incubate 2-10 min at RT and centrifuge 1 min at 8600 rpm.
24. Save aliquots of the RNA at -80°C.

4. Measuring the RNA concentration

Measure concentration using the Nanodrop
1 A260 unit RNA = 40 µg/ml
Recommended amount for labeling is 10-20 µg

5. Control gel (Optional)

- Rinse casting tray and the comb with 3% H_2O_2
- Pipette 150 µl 1 M Guanidine thiocyanate in a casting tray. (Fw=118,2 118 mg/ml prepare fresh).

  This will denature the RNA and gives better separation.
- Add 30 ml Agarose (1.5 % in TBE) with 2 µl EthBr *(5mg/ml)*/ 100 ml to the tray and mix using the comb.

  Use the same Agarose as for DNA gels.
- Load the gel :
  - 1.0 µl totRNA
  - 2.0 µl MQ water
  - 4.0 µl formamide
  - 1.0 µl loading dye (for RNA)
- Use normal DNA reference marker.
- Run at 8 V/cm (30mA) in 1x TBE buffer (same as DNA electrophoresis buffer)

Sometimes it is advisable to concentrate your RNA:

add: 1/10 volume of 1M NaAc (pH 4.5) and 2.5 volume cold EtOH

large volumes: o.n. – 20 °C

small volumes : 10' – 80 °C

6. Agilent

- Dilute 1 ul of sample with 50 ul of MiliQ DEPC to get a concentration of 20-200 ng/µl
- Use 1 ul diluted RNA sample to check quality on the Agilent BioAnalyser
- Ratio 23S:16S:~2.0

7. Materials.

acid phenol, RNA grade
Sigma P4682

macaloid:

- **Suspend 2 g. macaloid in 100mlTE**
- **Boil 5' and cool to RT**
- **Sonicate, by burst, until macaloid gels**
- **Centrifuge and resuspend in 50 ml TE pH8**
- **Store at 4°C**

Bentone MA (rheological additive)
Rheox Inc.
Nettlehill Road, Livingston
West Lothian
EH54 5 DL Scotland
UK

Solution : All solution should be treated with diethyl pyro-carbonate (DEPC). Add 100µl DEPC/100 ml solution, incubate over night at 37°C and autoclave for 15 minutes.

Always wear gloves when handling RNA.