

cDNA LABELING FOR MEMBRANE ARRAYS

Purpose: to make ^{33}P labeled cDNA from RNA template, using ^{33}P dCTP, for use as a probe for cDNA arrays

Materials Needed:

- 1-10 ug total RNA
- Oligo-dT 12-18 mer oligonucleotide (Invitrogen, cat# polyT.GF)
- DEPC H₂O
- Superscript II Reverse Transcriptase (Invitrogen, cat# 18064-014)
- 5X 1st strand buffer (Invitrogen, cat# 18064-014)
- 0.1M DTT (Invitrogen, cat# 18064-014)
- 3 dNTP's, 100mM each, dATP, dTTP, dGTP (Pharmacia cat#27-20X0-0)
- RNaseOut (Invitrogen, cat# 10777-019)
- 1.7 or 0.65ml eppendorf tubes
- ^{33}P alpha-dCTP Isoblue (ICN, cat# 58430)
- 0.5M EDTA solution
- 0.1N NaOH solution
- 1M Tris-HCl pH 8.0
- Biospin P-30 spin columns (Bio-Rad, Cat# 732-6223)

Preparation:

1. Mix 1ug of Oligo-dT oligonucleotide and 5-20ug of total RNA in a 1.5ml eppendorf tube.
2. If the volume needed to give 5ug of RNA is greater than 15ul, dry down the mixture in a speedvac.
3. Bring up total volume to 15ul with DEPC H₂O.
4. Heat to 65°C for 10 minutes.
5. Place on ice 2 minutes.

Procedure:

1. Prepare enough master mix to be distributed between your samples.

Labeling Reaction Master Mix **recommend making 10% more master mix than needed to account for differences in pipette calibration

REAGENT	Vol for 1 rxn	Vol for rxns
5X first strand buffer	8ul	ul
0.1M DTT	4ul	ul
Mix of 3 dNTP's minus dCTP	4ul	ul
RNaseOUT	1ul	ul

H2O	1ul	ul
SuperScript II Rev Transcriptase	2ul	ul
Total Volume	20ul	ul
Total Vol Master Mix/tube aliquoted to sample tubes	20ul	ul

2. Aliquot even amounts of master mix into each tube already containing 15ul RNA/Oligo-dT.
3. In the fume hood, add 5-10ul ³³P alpha-dCTP, depending on the reference date of the isotope, to each tube.
4. Incubate at 42 degrees 35 minutes.
5. Add an additional 2 ul SSII RT and incubate at 42 degrees 35 minutes.

Removal of RNA

6. Add 5ul 0.5M EDTA.
7. Add 10ul 0.1N NaOH.
8. Incubate at 65 degrees for 30 minutes.
9. Add 25ul 1M Tris-HCl pH 8.0.

Purification

10. Prepare a Biospin P-30 spin column/ as per manufacture's specifications.**
11. Load sample on column/spin.
12. Recover ~75ul sample. Count 1ul.

****BioSpin-30 Protocol.**

- a. Quick spin column to settle resin.
- b. Remove cap, break off bottom.
- c. Place in 2ml reservoir tube.
- d. Spin 1K RPM (#6) for 2 min.
- e. Discard effluent and repeat spin if all liquid is not removed from column.
- f. Place column in a new, labeled eppendorf tube.
- g. Apply sample to column (up to 75 ul)
- h. Spin column/tube 1K RPM (#6) for 4 min.
- i. Recover flow through, ~75 ul.
- j. Count 1 ul in scintillation counter and record results on labeling spreadsheet. Store labeled probes until you are ready to continue with the hybridization step.

Comments:

These are recommended labeling protocols. Variations of this or similar protocols may improve labeling efficiency depending on the input RNA or your hybridization system.

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For frequently asked questions go to the following address:

<http://www.grc.nia.nih.gov/branches/rrb/dna/protocolFAQs.htm>

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