NEB oligo-dT mRNA isolation Procedure

3-7-00

cws

Protocol Summary: Equilibrate Cellulose
Add salt to RNA
Denature and bind to cellulose
Remove, denature and bind again
wash cellulose 4 times in high salt
wash once in low salt
Elute twice

65-70 C heat source
1 mg of Total RNA

Prepare Resin:

- Remove oligo-dT and buffers from fridge and equilibrate to RT.
- Spin oligo-dT aliquot in microfuge, 10 sec. (2000 to 5000 x g)
- Remove sup.
- Add 200 ul Wash Buffer, mix thorooughly.
- Spin 10 sec
- Remove sup.

Prepare RNA

- Prepare 1 mg of Total RNA in 450 ul TE
- Add 50 ul 5 M NaCl

- Denature at 65 C, 5 minutes
- Chill on ice
- Add RNA to oligo-dT Mix thoroughly
- Incubate 5 min. at RT with gentle agitation
- Spin 10 seconds
- Remove Sup to original tube
- Repeat Denaturation and binding
- Spin 10 sec
- Remove sup to original tube, store on ice
- Add 400 ul Wash Buffer
- Agitate by hand to resuspend beads
- Transfer to column reservoir with 1 ml pipet
- Incubate 2 min with gentle agitation
- Spin 10 sec
- Remove sup to new tube
- Repeat wash 3 times
- Add 400 ul **Low Salt Buffer**
- Spin 10 sec
- Take column to new tube
- Add 200 ul warm **Elution Buffer**
- Incubate 2 min with gentle agitation
- Spin 10 sec
- Repeat elution with 200 ul fresh **Elution Buffer**
- Incubate 2 min with gentle agitation
- Spin 10 sec