

Affymetrix GeneChip Resource

ds-cDNA Cleanup Protocol

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A. Reagents Required:

DEPC-treated water (Ambion)

GeneChip sample cleanup module (Affymetrix)

Ethanol 96-100%

B. Equipments and supplies:

Microcentrifuge with 1.5 ml rotor.

Micropipettors

Aerosol-Barrier tips

Vortex mixer

Powder-free gloves.

1.5 ml microcentrifuge tubes.

C: PROCEDURE:

Important Notes:

- * GeneChip cleanup module is stored at 4^o C. Remove from the refrigerator, open the box and keep on the lab bench for at least 30 minutes to allow the temperature of the reagents to come to Room Temperature.
- * cDNA wash buffer is supplied as a concentrate. Before using for the first time, add 24 ml of ethanol (96-100%) as indicated on the bottle to obtain working solution. Check mark the bottle with black marker to indicate that ethanol has been added which will avoid the confusion.
- * All steps of the protocol should be performed at room temperature. During the procedure, work without interruption.

1. Transfer each cDNA sample from the 0.2 ml to a 1.5 ml tube. Add 600 μ l

cDNA binding buffer to the 162 µl final double s-stranded cDNA synthesis preparation. Shake tube vigorously by hand and then vortex thoroughly for 5 seconds.

2. Check that the color of the mixture is yellow (similar to cDNA binding buffer without the cDNA synthesis reaction).

Note: If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate pH 5.0 and mix. The color of the mixture will turn to yellow.

3. Apply 500 µl of the sample to the cDNA cleanup spin column sitting in a 2 ml collection tube and centrifuge for 1 minute at $\geq 10,000$ rpm.
4. Reload the spin column with the remaining mixture (262 µl). Transfer the flow-through to the original tube. Centrifuge the spin column as above.
5. Transfer the flow-through to the original tube. (This way we are saving the sample in case there is a problem with the column). Transfer spin column into new 2 ml collection tube. Pipet 750 µl cDNA wash buffer onto the spin column. Centrifuge for 1 minute at $\geq 10,000$ rpm.

Note: cDNA wash buffer is supplied as a concentrate. Ensure that ethanol is added to the cDNA wash buffer before use.

6. Open the cap of the spin column and centrifuge for 5 minutes at maximum speed. Discard flow-through and collection tube.

Note: Place column into the centrifuge using every second bucket. Position caps over the adjoining bucket so that they are oriented in the opposite direction to the rotation (i.e., if the microcentrifuge rotates in a clockwise direction, orient the caps in a counterclockwise direction). This avoids damage of the caps.

7. Leave the tubes on the bench top for 5 minutes with caps open to ensure that all the leftover ethanol is evaporated and the membranes are completely dry.
8. Transfer spin column into a 1.5 ml collection tube, and pipet 14 µl of cDNA elution Buffer **directly onto the spin column membrane**. Incubate for 2 minutes at room temperature and centrifuge 1 minute at maximum speed to elute.

Note: Ensure that the cDNA buffer is dispensed directly onto the membrane. The average volume of eluate is 12 µl from 14 µl elution buffer.

9. After cleanup the cDNA can be stored at -20° or used immediately for Biotin-labeled cRNA synthesis (IVT reaction).

E: REFERENCES:

* AFFYMETRIX GeneChip expression Analysis Technical manual

