DIG-LABELED OLIGO

MATERIALS
Chroma-spin TE-10 columns (Clonetech catalog # 636066)
DEPC Water
DIG-Labeling Kit (Roche catalog # 03 353 575 910)
Pipettes/Pipette tips
Samples

NOTES
1. Prepare your samples on ice.
2. Store your labeled oligos at -20°C.

PROCEDURE DIG Oligonucleotide 3' –End Labeling Kit, 2nd Generation Protocol (Roche Applied Science catalog # 03 353 575 910)

1. Prepare the sample(s). (10 µL)
   Control = 5 µl DEPC water + 5 µl control (vial 5 from DIG Labeling Kit)
   Sample-LNA = 1 µl oligo(@ 100pmol/µL) + 9 µl sterile DEPC water
2. Add the following to each sample on ice:
   4 µL 5X Reaction Buffer (vial 1)
   4 µL $CoCl_2$—solution (vial 2)
   1 µL DIG-ddUTP solution (vial 3)
   1 µL Terminal Transferase (vial 4)
3. Mix and centrifuge briefly.
4. Incubate the samples at 37°C for 15 minutes, then place on ice.
5. Stop the reaction by adding 2 µL 0.2 M EDTA (pH 8.0) (Fisher Scientific catalog # BP120-500).
6. Dilute each labeled probe to 50 µL total volume.

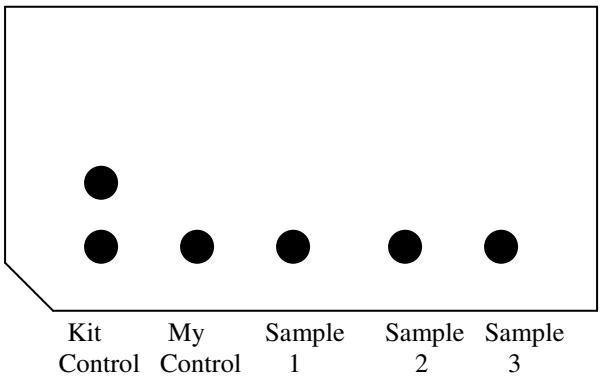
Use the Chroma Spin + TE-10 (Clonetech catalog# 636066) columns to purify the DIG-labeled oligo.

7. Invert the chromaspin column to resuspend the gel matrix completely.
8. Holding the column upright, grasp the break-away end between your thumb and index finger and snap off.
9. Place the end of the spin column into one of the 2-ml microcentrifuge (collection) tubes provided, and lift off the top cap.
10. Centrifuge at 700 x g for 5 minutes (2100 RPM).
11. After centrifugation, the column matrix will appear semi-dry. This step purges the equilibration buffer.
12. Remove the spin column and the collection tube from the rotor, and discard the collection tube and column equilibration buffer.
13. Place the spin column into the second 2-ml microcentrifuge tube. Carefully and slowly apply the sample to the center of the gel bed's flat surface. Do not allow any sample to flow along the inner wall of the column.
14. Centrifuge at 700 x g for 5 minutes (2100 RPM).

15. Remove the spin column and the collection tube from the rotor and detach them from each other. The purified sample is at the bottom of the collection tube.

Confirm labeling with a dot blot on a nylon membrane.

Goal



16. Dilute DIG-labeled samples, including the kit control, 1: 100 in sterile water.
17. Trim one corner of the nylon membrane as a reference point.
18. Apply 1 µL of the kit control, and 1 µL of each sample in a recognizable pattern (see above picture as a suggestion).
19. UV Crosslink the oligos to the membrane.
20. Follow the Roche DIG Wash and block buffer (Roche Applied Science catalog# 11 585 762 001) protocol for detection using Anti-DIG AP as your detector.
21. Place the membrane (RNA or DNA side up) in 100 mL Wash Buffer for 5 minutes, incubating at room temperature with rotation. Decant off the wash buffer.
22. Incubate the membrane with rotation @RT for 30 minutes in 100 mL Blocking solution. Decant off the blocking solution.
23. Add 1 µL of Anti-DIG AP (Roche Applied Science catalog # 11 093 274 910) to 20 mL of Blocking solution. This is your Antibody Solution.
24. Incubate the membrane with rotation @RT for 30 minutes in 20 mL Antibody solution.
25. Incubate the membrane with rotation @RT for 15 minutes in 100 mL Wash Buffer. Repeat step #27.
26. Incubate the membrane with rotation @RT for 5 minutes in Detection Buffer.
27. Place the membrane on a piece of clear plastic wrap and apply 1 mL CDP-Star. Incubate 5 minutes.
28. Drain off excess CDP-Star solution (Roche Applied Science catalog # 12 041 677 001) and cover the membrane with clear plastic wrap. Be sure not to let the membrane dry out.
29. Expose the membrane to film and develop.

SOLUTIONS

1X Block Buffer 100 mL (Roche Applied Science catalog # 11 585 762 001)

10 mL	10X Maleic Acid Buffer from Roche Kit
10 mL	10X Block Solution from Roche Kit
80 mL	DEPC Water

- Always apply Maleic Acid Buffer to DEPC water before adding Solution.

DEPC Water (1 Liter)

1 mL	DEPC (Sigma catalog # D5758)
1 L	Sterile ultrapure water

- Autoclave.

Detection Buffer (100 mL) (Roche Applied Science catalog # 11 585 762 001)

10 mL	10X Detection Buffer from Roche Kit
90 mL	DEPC Water

1X Wash Buffer (100 mL) (Roche Applied Science catalog # 11 585 762 001)

10 mL	10X Wash Buffer from Roche Kit
90 mL	DEPC water