^{32}P Radioactive Probe Synthesis and Preparation

Bio-protocol Editor*

Bio-protocol LLC, Sunnyvale, CA 94087, USA
*For correspondence: team@ed.bio-protocol.org

[Abstract] To probe for a specific mRNA species by Northern blot, RNA from the agarose/formaldehyde gel needs to be transferred to a nylon membrane. RNA is detected by hybridization using a labeled probe. The probe is a DNA or RNA molecule that is chemically or radioactively labeled. In this protocol synthesis and preparation of a [^{32}P]-dCTP-labeled probe is described.

Materials and Reagents

1. Amersham Megaprime DNA labeling system (GE Healthcare Life Sciences, catalog number: RPN 1606)
2. Amersham Microspin G-50 Columns (GE Healthcare Life Sciences, catalog number: 27-5330-01)
3. dCTP-apha-^{32}P 3000 Ci/mmol (PerkinElmer)
4. TE buffer
5. EDTA

Equipment

1. Scintillation counter
2. Bench-top centrifuge
3. Heat block
4. 37 °C incubator

Procedure

A. Labeling reaction

1. Place 25 ng of template in 28 µl TE or water in tube. Add 5 µl of primer solution from kit.
2. Denature by incubating at 100 °C for 5 min.
3. Spin tube briefly to bring contents of tube to bottom.

4. At room temperature, add 10 µl of labeling buffer, 5 µl of dCTP, and 2 µl of enzyme.
5. Mix and incubate at 37 °C for 10 min.
6. Stop reaction by addition of 5 µl of 0.2 M EDTA.

B. Probe purification

1. Prepare column by vortexing to resuspend matrix. Loosen cap ¼ turn and insert into 1.5 ml screw cap tube.
2. Spin 1 min at 735 *x g*. Start timer and centrifuge simultaneously.
3. Place column in a new 1.5 ml screw cap tube and slowly apply 50 µl of sample to center of column matrix.
4. Do not disturb the column bed. Do not let sample flow around the sides of the bed.
5. Spin column for 2 min at 735 *x g*.
6. Purified sample is now at the bottom of the tube.
7. Heat to 100 °C and chill on ice prior to adding to hybridization.
8. Count 1 µl of probe in scintillation counter.

Acknowledgments

This protocol has been modified and adapted in the Espenshade Lab, Johns Hopkins School of Medicine. Funding to support different projects that have used this protocol has come from NIH – National Heart, Lung, and Blood Institute, National Institute of Allergy and Infectious Diseases, the Pancreatic Cancer Action Network, and the American Heart Association.

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