

PROBE LABELING FOR SOUTHERN BLOT ANALYSIS

RadPrime DNA Labeling System (Invetrogen #18428-011)

1. Add H₂O to 25 ng of DNA template to a final volume of 21 μ l. For genomic Southern, add 1 μ l of λ /H3 marker (1/100 dilution of 100 ng/ μ l).
2. Denature at 99°C for 5 min and immediately chill on dry ice/EtOH.
3. Add 3 μ l of dATP/dGTP/dTTP mix (0.5 mM each).
4. Add 20 μ l of 2.5 x Buffer.
5. Add 5 μ l of isotope.
6. Add 1 μ l of Klenow.
7. Mix, and incubate at 37°C for 30~60 min.
8. Add 5 μ l STOP solution to stop reaction.
9. Add 10 μ l H₂O to a total volume of 65 μ l.
10. Toward the end of step 7, prepare Bio-Spin 30 column (BioRad, #732-6202).
 - a. Invert the column several times to resuspend the settled gel. Snap off the tip and place column in a 2.0 ml microcentrifuge tube. Remove cap to allow buffer to pass through the column.
 - b. Spin at 1,000xg (or 3,000 rpm in our bench-top centrifuge) for 2 min.
11. Place the column in a clean 1.5 ml microcentrifuge tube. Carefully add the labeling reaction 65 μ l directly to the center of the column.
12. Centrifuge the column for 4 min at 1,000xg.
13. Use half of the probe for 10 ml Hybridization buffer.