

CirmBlots

A Technologies Advancement Company

Multiple Tissues Northern Blots
Trouble Shooting Guide
TS-3001
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For Research Use Only

CirmBlots Troubleshooting Guide

A. High background

Lengthening the time of low or high stringency washes rarely decreases high background signals. Remove the probe from the blot as you would for reprobing. Monitor the process with a Geiger counter to verify cpm is reduced to almost background levels. If there is still a high background, store the blot covered in plastic wrap at -20°C for 2-3 half lives. The half life of ^{32}P is 14 days.

High background can occur for several reasons:

- Unincorporated [^{32}P] dNTPs are not fully removed from the probe.
Check the ratio of TCA-precipitable cpm to total cpm in the final probe preparation. Adjust chromatography conditions to achieve a ratio of >95%. We also recommend purifying your hybridization probe by exclusion chromatography using a CHROMA SPIN-100 Column
- Average size of the DNA probe is too large.
Optimal sizes range from 200-800 nucleotides. Increase the ratio of random primers to template DNA
- Concentration of probe in hybridization solution is too high.
For DNA probes, do not exceed 2×10^6 cpm/ml. For oligonucleotide probes, do not exceed 5×10^6 cpm/ml.

B. Hybridization signals absent or very weak

If signals are not generated after 1-2 days of x-ray film exposure, the hybridization probe may have a low specific activity. Make a new probe with fresh ^{32}P .

The specific activity of your probe should always be $>5 \times 10^8$ cpm/ μg , and, for maximum sensitivity $>1 \times 10^9$ cpm/ μg . If specific activity is still low upon repeating the labeling, you may be using too little DNA. We use 25-50 ng measured from the OD_{260} of a concentrated stock. If you have a small quantity of DNA, electrophorese the amount you have been using on an agarose gel, next to a known amount of DNA markers and estimate the amount of probe DNA. If a clear ethidium bromide-stained band is not observed, you have less than 25-50 ng, and therefore, should use 2-3 times more DNA for probe labeling. If this fails, optimize labeling conditions with a known amount of control probe such as β -actin.

C. Probe not fully homologous to target

If you are using a cross-species probe, you may need to reduce the stringency of your final wash by using Wash Solution 1 instead of Wash Solution 2 and decreasing the temperature by 5-10° C. When using a synthetic oligonucleotide probe, ensure that the probe is completely homologous to the target.

D. Inability to strip and reprobe

If you are unable to reprobe the blot, the membrane may not have been stripped completely or may have been allowed to dry or partially dry. If a membrane is allowed to even partially dry, subsequent removal of the probe may be impossible. To prevent drying after your final wash, shake off excess solution with forceps (do not blot-dry) and wrap the blot immediately with plastic wrap. When reprobing, uncover the blot, immediately place it in heated sterile water containing 0.5% SDS, and follow the rest of the protocol provided for removing probes. If the membrane has not partially dried, see Section A above.

E. Signal Decreases after two reprobings

This problem is especially common for low abundance genes. You may not see signals as strong as those observed for high-abundance transcripts such as β -actin.