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## PRODUCT TECHNICAL & APPLICATION NOTES

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### Application notes: Hybridization protocol tips

Hybridization techniques which involve the detection of membrane bound molecules are common place in most laboratories and include Northern (RNA), Southern (DNA) and Western (Proteins) blot. These may come in the form of molecules transferred from a gel, slot or dot blots where they are spatially placed on top of the membrane using a pipette or specialized manifold, or by colony/plaque adhesion.

During the hybridization process, while larger volumes wash buffers of are used, small volumes of probe and detection solutions are desired, all requiring a variety of temperatures. Below are some useful tips which improve the quality of your results.

**Hybridization vessels:** To optimize your results use specially designed hybridization incubators with rotating bottles. These offer many advantages including; reduced reagent volume, even membrane Hybridization, multiple membranes/vessel, easy probe recovery; temperature accuracy and reproducibility, safe liquid transfer.

**Multiple membranes:** When a large number of blots are to be hybridized with the same probe, time and reagents can be saved by placing them together. To do this, pre-warm the first reagent and place it in the pre-warmed incubation vessel before adding the membranes one by one, always ensuring that each one is fully coated before adding the next. To optimize probe: target accessibility use separation membranes between each blot.

**Improving signal: noise ratios:** One of the most difficult aspects of membrane hybridization is to keep a clean particulate free blot with a low background, while still retaining a good signal.

- **Temperature:** Chose the correct temperature. The easiest way to decrease the background during RNA and DNA blot hybridization procedures is to increase the temperature at which the probe and initial post-hybridization steps are carried out. As a guide, for a longer DNA probe (e.g. 200bp) start with 65C and for an oligonucleotide probe try 50C. For Protein detection, some primary antibodies are more specific when hybridized overnight at 4C rather than a few hours at room temperature.
- **Probes & antibodies:** Take care to keep stocks of probes and detection reagents at the recommended temperatures. For non-radioactive techniques, re-using probe and antibody Hybridization solutions can lead to significant improvements of signal (store antibodies ones at 4 °C and DNA at -20C). When using antibodies, give the stock tubes a quick spin in a microcentrifuge and pipette from the top of the liquid to remove background causing precipitants.
- **Clean equipment:** Cleanliness plays an important part in membrane hybridizations. Always wear gloves, handle blots with forceps and avoid dusty environments. For non-radioactive detection techniques, microwave the reaction vessels with some water in prior to re-use as this acts to remove residues which can cause serious background problems.
- **Buffers:** Only use high quality reagents and ultra-pure water. When making up stocks of large volume buffers such as SSC, pass them through 0.45 μm filters to remove particulates. If using formamide, make sure it has been deionized first.
- **Crosslinking:** Using a UV-Crosslinker to irreversibly bind your nucleic acids to the membrane is faster and much more efficient than traditional baking resulting in a higher signal.