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SIGMA®

ProductInformation

PERFECTHYB™ PLUS

Product No. **H 7033**
Technical Bulletin No. MB-610
Store at room temperature

TECHNICAL BULLETIN

Product Description
PerfectHyb™ Plus hybridization buffer has been optimized to yield maximum signal with minimum background in hybridizations as short as 1-2 hours. PerfectHyb Plus has been formulated to work in any hybridization protocol, utilizing any type of probe, and on any type of membrane (positively charged or neutral nylon and nitrocellulose).

Although signal continues to increase over time (see Figure 1), signal equivalent to overnight hybridizations using conventional hybridization buffers is achieved within 2-3 hours using PerfectHyb Plus. This allows the user to tailor the length of hybridization according to the needs of the experiment. For example, when conducting simple hybridization experiments such as screening PCR products by Southern blotting, a 30 to 60 minute hybridization is likely sufficient. If increased sensitivity is desired, the researcher can extend the hybridization to 2 to 3 hours to achieve signal equivalent to that observed in overnight hybridizations utilizing conventional hybridization buffers. To maximize sensitivity, the hybridization can be extended overnight to obtain a 2 to 5 fold increase in signal over conventional buffers without increasing background levels.

Many hybridization buffers, which yield increased rates of hybridization, are hampered by restrictions on the types or amounts of probe that can be used. PerfectHyb Plus yields high signal to noise ratios with both radioactive and non-radioactive labeled probes. Any type of probe can be used including single-stranded or double-stranded DNA, RNA, and oligonucleotides. In addition, PerfectHyb Plus is not affected by the addition of excess or uncleaned probes.

Although PerfectHyb Plus will work in any hybridization protocol, the following procedures are recommended for maximum sensitivity.

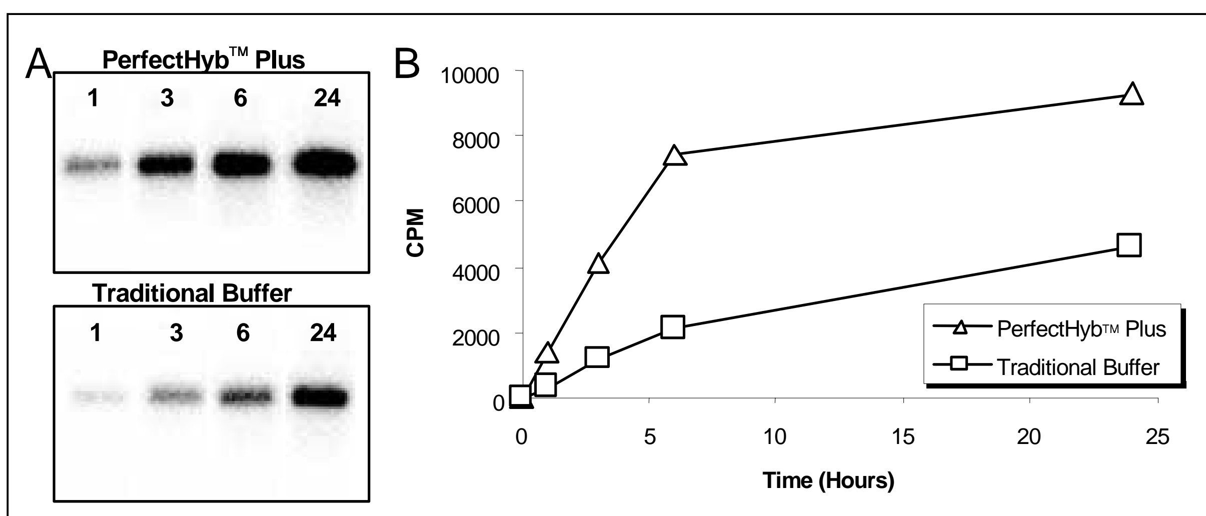

Figure 1: Northern blot hybridizations comparing PerfectHyb Plus with a traditional hybridization buffer (0.25 M sodium phosphate, pH 7.2, 7% sodium dodecyl sulfate (SDS), and 2 mM EDTA). (A) Northern blots with 2 µg total mouse kidney RNA per lane were hybridized for the indicated amount of time with ~5×10^5 cpm ^{32}P-labeled β-actin riboprobe per milliliter. After hybridization, blots were washed to high stringency, exposed and analyzed. (B) Counts per minute (cpm) obtained from blots shown in (A) plotted versus length of hybridization in hours.

Precautions and Disclaimer
PerfectHyb Plus is for laboratory use only, not for drug, household or other uses.

Storage/Stability
All materials may be stored at room temperature. Solutions have a shelf life of 1 year upon receipt. If a small amount of precipitate forms, heat the solution to 60-80 °C, mixing periodically, until dissolved. Once completely dissolved, invert to mix and store at room temperature.

Reagents that May be Required But are Not Provided
(Sigma product numbers are given where appropriate)

- 20X SSC (3 M sodium chloride, 0.3 M sodium citrate, pH 7.0) (Saline-sodium citrate buffer), Product No. S 6639
- 10% SDS (Sodium dodecyl sulfate), Product No. L 4522
- Molecular Biology Grade Water, Product No. W 4502

Preparation Instructions
Buffer Preparations

- Low Stringency Wash Buffer (2X SSC, 0.1% SDS)
  To 500 ml molecular biology grade water, add 100 ml of 20X SSC stock solution and 10 ml of 10% SDS stock solution. Adjust volume to 1 L with water.
- High Stringency Wash Buffer (0.5X SSC, 0.1% SDS)
  To 500 ml of molecular biology grade water, add 25 ml of 20X SSC stock solution and 10 ml of 10% SDS stock solution. Adjust volume to 1 L with water.
- Ultra-High Stringency Wash Buffer (0.1X SSC, 0.1% SDS)
  To 500 ml of molecular biology grade water add 5 ml of 20X SSC stock solution and 10 ml of 10% SDS stock solution. Adjust volume to 1 L with water.

Procedure
Hybridization Procedure for Radioactive and Non-radioactive Probes

1. Pre-hybridize membranes for at least 5 minutes at the appropriate hybridization temperature (see below). Longer pre-hybridizations are not necessary.

Probe Type	Hybridization Temperature[a]
DNA	68 °C
RNA	68 °C
Oligonucleotides	37-45 °C

[a] Temperatures for probes of irregular G+C content must be determined empirically.

Note 1: Sufficient volumes of hybridization solution must be used to ensure complete coverage of the membrane.
Note 2: Sigma has found that a blocking agent is not necessary when using PerfectHyb Plus. If a blocking agent is preferred, the addition of single stranded DNA at 0.1 mg/ml is recommended.

Hybridization Vessel	Recommended Volumes
Heat-sealable bags	100 μl/cm^2
50 ml conical tubes	5 ml
Small Hyb tube	7 ml
Large Hyb tube	10 ml

2. For double-stranded DNA probes, denature by heating probe to 100 °C for 10 minutes. Quick chill on ice for 2 minutes. Single-stranded DNA and RNA probes do not require denaturation prior to addition to the hybridization reaction.

3. Add denatured probe directly to pre-hybridization solution or add to fresh prewarmed PerfectHyb Plus and replace pre-hybridization solution with this mixture. For a radiolabeled probe, $\geq 1 \times 10^6$ cpm is usually sufficient to detect most targets.

4. Choose the length of hybridization to achieve the required sensitivity according to the table below and the graph shown in Figure 1.

Blotting Application	Required Sensitivity	Recommended minimum length of hybridization
Simple Southern blot[b]	Low	30-60 minutes
Genomic Southern blot	High	2-3 hours
Northern blot	High	2-3 hours
Colony/Plaque lifts	Low	30-60 minutes

[b] Blots screening plasmid preps or PCR products.

5. After hybridization is complete, discard probe and wash blots once for 5 minutes at room temperature in low stringency wash buffer.

6. For high stringency, wash twice for 20 minutes at hybridization temperature in high stringency wash buffer. For highest stringency, a final wash for 20 minutes at temperature with ultra-high stringency wash buffer may be added.

7. If radioactive probes were used, the blots are now ready to be exposed to film. Wrap blots in plastic wrap or seal in bags. Expose to X-ray film using an intensifying screen at –70 °C.

8. For non-radioactive probes, use colorimetric or chemiluminescent detection system according to manufacture's instructions.

Results

Troubleshooting Guide

Problem	Cause	Solution
Precipitate present in buffer	Storage temperature of buffer falling below room temperature.	Heat to 60-80 °C for 15 minutes, mixing periodically. Make sure the buffer is mixed well before use.
High Background	Non-specific binding of probe to target nucleic acids.	Add sheared, denatured salmon testis DNA (Product No. D 7656) to a final concentration of 100 μg/ml in prehybridization and hybridization solutions.
	Wash conditions not sufficiently stringent	Add the Ultra-High Stringency Wash step. Increase the temperature of the hybridization and/or washes.
	Exposure to film was too long	Shorten the exposure time to film.
	Concentration of enzyme conjugate in non-radioactive detection is too high.	Dilute the enzyme conjugate further. The specific dilution required for optimal signal to noise must be determined empirically.
Weak/Absent Signal	Probe was not labeled efficiently	Check that the specific activity of radiolabeled probes is $>5 \times 10^8$ cpm/μg. For non-radioactive probes, check the incorporation of hapten by spotting and detecting serial dilutions of probe in direct comparison to a known standard. If probes are not labeled well enough, remake and confirm adequate incorporation rates.
	Target nucleic acids are not present, have been degraded, or are too low for detection.	Run agarose gel electrophoresis to confirm nucleic acids are not degraded. Load more target nucleic acids for blotting. For Southern blots, up to 10 μg DNA can be loaded per lane. For Northern blots, up to 30 μg total RNA can be loaded per lane.
	Non-radioactive detection system is not working properly.	Confirm the enzyme/antibody conjugate is functioning properly by spotting and detecting the labeled probe on nylon membrane. If the enzyme/antibody conjugate is functional, check the chemiluminescent substrate by spotting the enzyme/antibody conjugate on a membrane and detecting with the substrate in question.

Related Products
All-in-One Nick Translation Labeling Mixes,
Product No. N 9155, N 8530, N 8405, N 9280
All-in-One Random Prime Labeling Mixes,
Product No. R 7522, R 9647, R 7022, R 9522
BioProbe Non-radioactive Labeling Kits
Chemiluminescent Detection Kit, Product No. LUM-1
5X Alkaline Phosphatase Reaction Buffer,
Product No. A 5957
1M Trizma-HCl, pH 7.4, Product No. T 2663
5 M Sodium Chloride (NaCl), Product No. S 5150
Tween-20, Product No. P 9416
1 M Magnesium Chloride ($MgCl_2$), Product No. M 1028
Streptavidin, Alkaline Phosphatase conjugate,
Product No. S 2890
Extravidin® Alkaline Phosphatase Conjugate,
Product No. E 2636
Avidin Alkaline Phosphatase Conjugate,
Product No. A 7294
NBT/BCIP Liquid Substrate, Product No. B 1911
CDP-*Star*™ Universal Alkaline Phosphatase Detection Kit, Product No. U-ALK
CDP-*Star*™ Chemiluminescent Substrate Solution,
Product No. C 0712
Neutral or positively charged BioBond™ membrane
BioMax Films

Reference
Sambrook, J., *et al.,* Molecular Cloning: A Laboratory Manual, (1989) second edition, Cold Spring Harbor Laboratory Press, New York

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