GE Healthcare

Amersham Rapid-hyb buffer

Rate enhanced hybridization buffer for use with radiolabeled nucleic acid probes

A major limitation of filter hybridization is speed: at commonly used probe concentrations (5–10 ng/ml), hybridization is usually conducted for at least 16 hours in order to detect single-copy gene sequences in mammalian DNA.

Analysis of the kinetics of filter hybridization has led to the development of Rapid-hyb™ buffer. This hybridization cocktail allows single copy mammalian genes to be detected after only a 2 hour hybridization with ^{32}P labeled probes. For many applications a 1 hour hybridization is sufficient.

Product Specification Sheet

Codes: RPN1635
RPN1636

Warning
For research use only.
Not recommended or intended for diagnosis of disease in humans or animals.
Do not use internally or externally in humans or animals.

Storage

Store at ambient temperature. Under these conditions the product is stable for at least 3 months.

Expiry

See outer packaging.

Components

- **RPN1635 - 125 ml:** Sufficient for 1000 cm^2 membrane.
- **RPN1636 - 500 ml:** Sufficient for 4000 cm^2 membrane.

Safety warnings and precautions

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Probe type

Rapid-hyb buffer is optimized for use with radiolabeled DNA, RNA or oligonucleotide probes. Rapid-hyb buffer contains chemical blocking agents. This removes the requirement for heterologous DNA to control non-specific binding of probes to the membrane.

When using ^{32}P labeled probes, removal of unincorporated nucleotides is unnecessary. For ^{35}S labels, unincorporated nucleotides should be removed prior to hybridization.

The buffer has been optimized for use with Hybond™-N+ nylon membranes and can be used for the analysis of DNA or RNA blots. The use of PVDF or nitrocellulose membranes is not recommended.

Protocol for membrane hybridization of radiolabeled probes using Rapid-hyb buffer

1. Pre-warm the rapid hybridization buffer to 65°C for DNA probe hybridizations, 70°C for RNA probe hybridizations or 42°C for oligonucleotide probes as appropriate.

Note: These temperatures are suitable for hybridization of probes of an average (G+C) content (40%). The optimal temperature for probes of unusual (G+C) content must be determined empirically.

2. Immerse the blot completely in the buffer and pre-hybridize with shaking at the appropriate temperature for at least 15 minutes.

Note: Pre-hybridization and hybridization can be performed in a heat-sealed plastic bag, in a plastic box, or in a glass bottle inside a hybridization oven. When using bags, 0.125 ml/cm^2 is recommended. For other containers, the volume of buffer must be sufficient to completely cover the membrane or high backgrounds may result.

3. For random primed or nick translated DNA probes: Denature the DNA probe at 95–100°C for 2–5 minutes and chill on ice.

4. Add sufficient volume of probe to the hybridization buffer to achieve the recommended final probe concentration (see other side), and mix to ensure uniform distribution of the probe.

Note: Do not add concentrated probe directly on to the membrane as localized background may result. 0.5–1 ml of the buffer used for pre-hybridization can be withdrawn for mixing with the probe. The mixture should then be added back to the hybridization container.

5. Hybridize with shaking for 1–2.5 hours at 65°C for DNA probes, 70°C for RNA probes. For oligonucleotides, hybridize for 30–60 minutes at 42°C.

Note: Using recommended probe concentrations, this incubation is sufficient to give sensitivities equivalent to those obtained using conventional hybridizations. For high target applications shorter hybridization times can be used.

6. Stringency washes:

Note: 20 x SSC = 3 M NaCl, 0.3 M Na3 citrate.

DNA or RNA probes: 20 minutes in 50 ml 2 x SSC, 0.1% (w/v) SDS at ambient temperature. 2 x 15 minutes in 50 ml 1.0–0.1 x SSC, 0.1% (w/v) SDS at 65°C.

Note: These washing conditions are suitable for DNA or RNA probes as described in note 1, but for DNA or RNA of unusual (G+C) content, the optimal washing conditions should be determined experimentally. Notably, for certain RNA probes, washing temperatures above 65°C may be necessary in order to attain the correct stringency (temperatures up to 70°C have been used).

Oligonucleotide probes: 20 minutes in 50 ml 5 x SSC, 0.1% (w/v) SDS at ambient temperature. 2 x 15 minutes in 50 ml 1.0–0.1 x SSC, 0.1% (w/v) SDS at 42°C.

7. Wrap the washed filter in SaranWrap™ and autoradiograph.

Note: For ^{32}P-labeled probes, autoradiograph at -70°C using two intensifying screens and pre-flashed film for maximum sensitivity. For ^{35}S-labeled probes, autoradiograph dried filters at ambient temperature, without SaranWrap.

Note: If the filter is to be re-probed, do not allow it to dry completely.

Recommended final probe concentrations

Random-prime labeled probes:	~2 ng/ml, add 25% of a standard 25 ng random prime labeling reaction per 5 ml.
Nick translated DNA probes:	~2 ng/ml, add 20% of a small scale 50 ng nick translation reaction per 5 ml. For a standard 0.5–1.0 µg reaction, a probe concentration of up to 10 ng/ml is required.
SP6/T7 generated RNA probes:	~6 ng/ml, add 25% of a standard 20 ml probe preparation per 5 ml.
Oligonucleotides:	10 ng/ml.

For high target applications, for example colony or plaque screens, lower probe concentrations can be used.

Note: Probe concentrations significantly in excess of those recommended (>10 ng/ml) can lead to increased backgrounds (particularly when using probes contained in cloning vectors).

Specification

Rapid-hyb buffer is tested by our quality control group to ensure that a single copy gene can be detected in human genomic DNA blots using a DNA probe at a concentration of ~2 ng/ml, labeled with ^{32}P using the Megaprime™ DNA labeling system, following a 2 hour hybridization at 65°C.

To pass the QC specification a band equivalent to 0.5 pg target must be visualized after a 16 hour exposure to Hyperfilm™-MP.

Related products

Nucleic Acid Labeling Systems

Megaprime DNA Labeling System		
For use with labeled dCTP	30 reactions	RPN1606
	60 reactions	RPN1607
For use with any dNTP	30 reactions	RPN1604
	60 reactions	RPN1605
Nick Translation Kit		
For use with labeled dCTP	20 reactions	N5000
For use with any labeled dNTP	20 reactions	N5500
Rediprime II DNA Labeling System	30 reactions	RPN1633
	60 reactions	RPN1634
Ready-To-Go™ DNA Labeling Beads (-dCTP)		27-9240-01

Membranes

Hybond-N+; positively charged nylon hybridization membrane

Products for Autoradiography

Hyperfilm range of X-ray film

Hypercassette™ range of cassettes for autoradiography

Sensitize™ preflash gun		RPN2051
TrackerTape™	10 sheets	RPN2050

An adhesive waterproof tape that phosphoresces to give a permanent written image on autoradiography film.

imagination at work

Legal

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