Product specification

Rapid-hyb buffer

Rate enhanced hybridization buffer for use with radiolabelled nucleic acid probes

RPN 1635 - 125ml
Sufficient for 1000cm^2 membrane

RPN 1636 - 500ml
Sufficient for 4000cm^2 membrane

Safety warnings and precautions

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.

We recommend that this product and components are 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. All chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

A major limitation of filter hybridization is speed: at commonly used probe concentrations (5-10ng/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 labelled probes. For many applications a 1 hour hybridization is sufficient.

Probe type

Rapid-hyb buffer is optimized for use with radiolabelled 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 labelled probes, removal of unincorporated nucleotides is unnecessary. For ^{35}S labels, unincorporated nucleotides should be removed prior to hybridization.

The buffer has been optimised for use with Hybond^{TM}-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.

GE imagination at work

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

Protocol

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.
2. Immerse the blot completely in the buffer and pre-hybridize with shaking at the appropriate temperature for at least 15 minutes.
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 below), and mix to ensure uniform distribution of the probe.

Notes

1. 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. 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, 0.125ml/cm^2 is recommended. For other containers, the volume of buffer must be sufficient to completely cover the membrane or high backgrounds may result.
4. Do not add concentrated probe directly on to the membrane as localized background may result. 0.5-1ml 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.

Recommended final probe concentrations:

Random-prime labelled probes:	~2ng/ml, add 25% of a standard 25ng random prime labelling reaction per 5ml.
Nick translated DNA probes:	~2ng/ml, add 20% of a small scale 50ng nick translation reaction per 5ml. For a standard 0.5-1.0µg reaction, a probe concentration of up to 10ng/ml is required.
SP6/T7 generated RNA probes:	~6ng/ml, add 25% of a standard 20ml probe preparation per 5ml.
Oligonucleotides:	10ng/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 (>10ng/ml) can lead to increased backgrounds (particularly when using probes contained in cloning vectors).

Protocol	Notes
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.	5. 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:	6. 20x SSC = 3M NaCl, 0.3M Na3 citrate.
DNA or RNA probes: 20 minutes in 50ml 2x SSC, 0.1%(w/v) SDS at room temperature. 2x15 minutes in 50ml 1.0-0.1x SSC, 0.1%(w/v) SDS at 65°C.	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 50ml 5x SSC, 0.1%(w/v) SDS at room temperature. 2x15 minutes in 50ml 1.0-0.1x SSC, 0.1%(w/v) SDS at 42°C.	
7. Wrap the washed filter in SaranWrap™ and autoradiograph.	7. For ^{32}P-labelled probes, autoradiograph at -70°C using two intensifying screens and pre-flashed film for maximum sensitivity. For ^{35}S-labelled probes, autoradiograph dried filters at room temperature, without SaranWrap. **Note: If the filter is to be re-probed, do not allow it to dry completely.**

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 ~2ng/ml, labelled with ^{32}P using the Megaprime™ DNA labelling system, following a 2 hour hybridization at 65°C.

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

Storage

Stable for at least 3 months when stored at room temperature.

Related products

Nucleic acid labelling systems

Megaprime DNA labelling system		
For use with labelled dCTP	30 reactions	RPN 1606
	60 reactions	RPN 1607
For use with any dNTP	30 reactions	RPN 1604
	60 reactions	RPN 1605
Nick translation kit		
For use with labelled dCTP	20 reactions	N 5000
For use with any labelled dNTP	20 reactions	N 5500
3' End labelling kit	20 reactions	N 4020
Rediprime II	30 reactions	RPN 1633
	60 reactions	RPN 1634

Labelled nucleotides

GE Healthcare supplies a wide range of [^{32}P]-and[^{35}S]-labelled nucleotides for use in all types of labelling reactions. See catalogue for details or contact your local GE Healthcare representative.

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		RPN 2051
TrackerTape™	10 sheets	RPN 2050

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

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