For general laboratory use.
FOR *IN VITRO* USE ONLY.

DIG Easy Hyb

Hybridization solution for nucleic acid blots with digoxigenin-labeled probes

Cat. No. 1 603 558 500 ml

Version 2, March 2003

Store at 15 - 25° C

1. Product overview

Contents

Bottle	Label	Content
1	DIG Easy Hyb	500 ml, ready to use solution DNase and RNase free

Product description

DIG Easy Hyb is non-toxic and does not contain formamide, yet the hybridization temperature should be calculated with the same equation that is used for buffers containing formamide, 50%.

Application

DIG Easy Hyb can be applied for all types of nucleic acid blot hybridizations.

It is especially designed for the use with digoxigenin (DIG)-labeled nucleic acid probes to targets bound to nylon membrane.

Prehybridization

Prehybridization with DIG Easy Hyb is performed for 15-30 min at the appropriate hybridization temperature.

Hybridization

DIG Easy Hyb can drastically reduce hybridization times to only 1-6 hours, depending on the type of hybridization. Only for high sensitivity requirements, overnight (12-16 h) hybridization is recommended.

Application	Recommended hybridization time
DNA fingerprinting (multiple locus probes)	2-4 hours
DNA fingerprinting (single locus probes)	overnight
Colony/plaque hybridization	>4 hours
Single-copy gene detection in human gemomic blots	overnight
RNA:RNA hybridization	6 h - overnight
Oligonucleotide probes	1-6 hours

Storage/ stability

The unopened bottle is stable at 15-25°C through the control date printed on the label.

Storage of DIG-labeled probes in DIG Easy Hyb

Probes labeled with digoxigenin can be stored in DIG Easy Hyb at −15 to −25°C and be re-used several times, after denaturing at 68° C prior to use**, do not boil**.

Note: Storage and reuse of RNA probes in DIG Easy Hyb is generally not recommended and depends on probe inherent stability and RNase-free handling conditions.

2. Procedures and required materials

2.1 Before you begin

Additional equipment required

Hybridization can be performed in temperature resistant, sealable

- Hybridization bags* (Cat. No. 1 666 649)
- plastic or glass boxes
- petri dishes
- roller bottles

2.2 DNA : DNA hybridization

Hybridization temperature

The appropriate hybridization temperature is calculated according to GC content according to the following equation:

$T_m = 49.82 + 0.41$ (% G + C) - (600/l)
[l = length of hybrid in base pairs]

$T_{opt.} = T_m - (20 \text{ to } 25°C)$

(The given numbers of the equation were calculated according to a standard equation for hybridization solutions containing formamide, 50%.)

The actual hybridization temperature $T_{opt.}$ for hybridization with DIG Easy Hyb is 20−25° C below the calculated T_m value. $T_{opt.}$ can be regarded as a stringent hybridization temperature allows up to 18 % mismatches between probe and target. When the degree of homology of your probe to template is less than 80%, you should lower $T_{opt.}$ accordingly (approx. 1.4°C below T_m per 1 % mismatch) and also adjust the stringent washing steps accordingly (*i.e.* increase SSC concentration and lower washing temperature).

Example

For hybridization of human genomic DNA with a 100% homologous probe use 37−42°C, depending on the GC contents of the probe.

Procedure

In the following table the procedure for a DNA:DNA hybridization is described.

Note: Do not use open trays when working with DIG Easy Hyb buffer.

Step	Action
1	Pre-heat appropriate volume of DIG Easy Hyb (approx. 20 ml/100 cm²) to hybridization temperature.
2	Incubate the blot for 15-30 min with gentle agitation. ***Note***: The membrane should be well immersed and covered with DIG Easy Hyb.
3	Denature DIG-labeled DNA probe (5-25 ng/ml hybridization solution) by boiling for 5 min and rapidly cooling on ice-water.
4	Add to pre-heated DIG Easy Hyb (at least 3.5 ml/100 cm² membrane) and mix well but avoid foaming (bubbles may lead to background).
5	Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane. ***Note***: Do not add concentrated probe directly to avoid ocalized background.
6	Incubate with gentle agitation for at least 6 h at hybridization temperature ***Note***: For single copy detection we recommend o/n incubation.

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2.3 RNA : RNA hybridization

Hybridization temperature

For RNA:RNA hybridization in general 68° C is the recommended hybridization temperature. The actual hybridization temperature may have to be adjusted depending on the GC content, and homology of probe to target.

Procedure

In the following table the procedure for a RNA:RNA hybridization is described.

Note: Do not use open trays when working with DIG Easy Hyb buffer.

Step	Action
1	Pre-heat appropriate volume of DIG Easy Hyb (approx. 20 ml/100 cm²) to hybridization temperature.
2	Incubate the membrane for 30 min with gentle agitation. ***Note***: The membrane should be well immersed and covered with DIG Easy Hyb.
3	Denature DIG-labeled RNA probe (100 ng/ml hybridization solution) by boiling for 5 min and rapidly cooling on ice-water.
4	Add to pre-heated DIG Easy Hyb (at least 3.5 ml/100 cm² membrane) and mix well but avoid foaming (bubbles may lead to background).
5	Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane. ***Note***: Do not add concentrated probe directly to avoid localized background.
6	Incubate with gentle agitation for at least 6 h at hybridization temperature ***Note***: For detection of rare mRNAs we recommend 16 h incubation time.

2.4 DNA : RNA hybridizations

Hybridization temperature

For DNA:RNA hybridization in general 50°C is the recommended hybridization temperature. The actual hybridization temperature may have to be adjusted depending on the GC content, and homology of probe to target.

Procedure

In the following table the procedure for a DNA:RNA hybridization is described.

Note: Do not use open trays when working with DIG Easy Hyb buffer.

Step	Action
1	Pre-heat appropriate volume of DIG Easy Hyb (approx. 20 ml/100 cm²) to hybridization temperature.
2	Incubate the blot for 30 min with gentle agitation. ***Note***: The membrane should be well immersed and covered with DIG Easy Hyb.
3	Denature DIG-labeled DNA probe (5-25 ng/ml for DNA-probes, 100 ng/ml for RNA-probes) by boiling for 10 min and rapidly cooling on ice-water.
4	Add to pre-heated DIG Easy Hyb (at least 3.5 ml/100 cm² membrane) and mix well but avoid foaming (bubbles may lead to background).
5	Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane. ***Note***: Do not add concentrated probe directly to avoid localized background.
6	Incubate with gentle agitation for at least 6 h at hybridization temperature. ***Note***: For detection of rare mRNAs we recommend 16 h incubation time

2.5 Hybridization with DIG labeled oligonucleotide probes

Hybridization temperature

The hybridization temperature is calculated as follows:

Calculate T_m of the oligonucleotide probe by summing up 4° C for each G and C and 2°C for each T or A.

Perform prehybridization and hybridization at 10°C below evaluated T_m.

Multiple locus fingerprinting probes

For multiple locus fingerprinting probes we recommend 2 to 4 h hybridization time. Unspecific competitor DNA like DNA, MB grade from fish sperm (Cat. No. 1 467 140) should be added at a concentration of 50 µg/ml.

Procedure

In the following table the procedure for hybridization with DIG labeled oligonucleotide probes is described.

Note: For tailed oligonucleotides add 0.1 mg/ml poly (A) and 5 µg/ml poly d(A) to the prehybridization and hybridization to prevent unspecific hybridization signals caused by the tails.

Do not use open trays when working with DIG Easy Hyb buffer.

Step	Action
1	Pre-heat appropriate volume of DIG Easy Hyb (approx. 20 ml/ 100 cm²) to hybridization temperature.
2	Incubate the blot for 30 min with gentle agitation. ***Note***: The membrane should be well immersed and covered with DIG Easy Hyb.
3	Hybridize with 0.1-2 pmol tailed oligonucleotide/ml of hybridization solution or 1-10 pmol of end-labeled oligonucleotide. Use at least 3.5 ml DIG Easy Hyb per 100 cm² of membrane.
4	Add to pre-heated DIG Easy Hyb (at least 3.5 ml/100 cm² membrane) and mix well but avoid foaming (bubbles may lead to background).
5	Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane.
6	Incubate with gentle agitation for 1-6 h at hybridization temperature. ***Note***: For detection of rare mRNAs we recommend 16 h incubation time.

2.6 Plaque/ colony hybridization

Additional reagents required

- 10% SDS (w/v)
- 20× SSC: 3 M NaCl, 0.3 M sodium citrate, pH 7.0

Hybridization temperature

The appropriate hybridization temperature is calculated according to G/C content and percent homology of probe to target DNA with the following equation:

$T_m = 49.82 + 0.41$ (% G+C) - (600/l)
[l = length of hybrid in bp]

$T_{opt.} = T_m$ - (20° to 25°C)

The actual hybridization temperature $T_{opt.}$ with DIG Easy Hyb is 20−25°C below T_m.

Procedure

- The following volumes are calculated for the use of a 275 ml volume roller-bottle.
- The hybridization temperature is given for a 100% homologous probe with 50% G/C content.
- Please make certain that the membranes do not stick to each other and are sufficiently covered with hybridization solution.

Note: Do not use open trays when working with DIG Easy Hyb buffer.

Step	Action
1	• Place 3 membrane discs (82 mm ∅) in a roller bottle and add 60 ml DIG Easy Hyb. • Prehybridize for 1 h at 42°C in a hybridization oven for roller bottles.
2	Denature the labeled probe (25 ng/ml hybridization solution) by boiling for 5 min at 95-100°C and rapidly place on ice.
3	Mix the denatured probe with DIG Easy Hyb, prewarmed to hybridization temperature (5-25 ng/ml).
4	Remove the prehybridization solution and add 6 ml of the probe/ DIG Easy Hyb mixture.
5	Incubate for 2 h at 42°C. ***Note***: The hybridization solution with the DIG-labeled probe is stable at -15 to -25°C for more than 12 months and can be reused several times when freshly denatured.

2.7 Immunogical Detection

The procedure for the immunological detection of DIG-labeled nucleic acids is described in the pack inserts of the DIG Luminescent Detection Kit (Cat. No. 1 363 514) or the DIG Wash and Block Buffer Set (Cat. No. 1 585 762).
These pack inserts are available from our website http://www.roche-applied-science.com.

2.8 Post Hybridization Washes, Stripping and Rehybridization

Post hybridization washes

Please find in the following table the procedure for the post hybridization washes.

Step	Action
1	Wash 2× 5 min in ample 2× SSC; SDS 0.1% at 15 - 25°C.
2	Wash 2× 15 min in 0.1 × SSC; SDS 0.1% at 68° C under constant agitation.

Stripping and rehybridization

Please refer to the following table.

Note: When stripping and rehybridization of blots is planned, the membrane should not dry off at any time.

Caution

Work in a fume hood

Step	Action
1 (only color detection)	Pre-heat dimethylformamide in a waterbath to 50-60°C and incubate the membrane until the color (NBT/BCIP) is washed off. ***Note***: DMF is volatile and can be ignited above 67°C.
2	Rinse membrane briefly in sterile double distilled water.
3	Wash for 2× 20 min in 0.2 M NaOH, SDS, 0.1% (w/v) at 37°C under constant agitation.
4	Equilibrate briefly in 2× SSC.
5	Prehybridize and incubate with second probe.

3.2 Reference

1 Itakura, K. et al. (1984) Annu. Rev. Biochem. **53**, 323

* available from Roche Applied Science

3.3 Related products

Kits

The use of DIG Easy Hyb is recommended in combination with the following DIG kits and replaces the therein mentioned hybridization solutions.

Product	Pack Size	Cat. No
DIG DNA Labeling Kit	40 labeling reactions	1 175 033
DIG RNA Labeling Kit	2 × 10 reactions	1 277 073
DIG Oligonucleotide 3'-End Labeling Kit	25 reactions	1 362 372
DIG Oligonucleotide Tailing Kit	25 reactions	1 417 231
DIG DNA Labeling and Detection Kit	25 labeling reactions and 50 blots (10× 10 cm^2)	1 093 657
DIG Nucleic Acid Detection Kit	40 blots (10× 10 cm^2)	1 175 041
DIG Luminescent Detection Kit for Nucleic Acids	50 blots (10× 10 cm^2)	1 363 514
DIG Wash and Block Buffer Set	1 set	1 585 762

Single reagents

Product	Pack Size	Cat. No.
Nylon membranes, positively charged	10 sheets (20 × 30 cm) 20 sheets (10 × 15 cm) 1 roll (0.3 × 3 m)	1 209 272 1 209 299 1 417 240
Nylon Membranes for Colony and Plaque Hybridization	50 filters (∅ 82 mm) 50 filters (∅ 132 mm)	1 699 075 1 699 083
DNA, MB-grade	500 mg (50 ml)	1 467 140
Hybridization bags	50 bags	1 666 649

For your further information:

Roche Applied Science offers a large selection of products for the non-radioactive labeling and detection of nucleic acids.

For a complete overview, please visit and bookmark our "DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection" Special Interest Site at http://www.roche-applied-science.com/DIG

How to contact Roche Applied Science

www.roche-applied-science.com

to order, solve technical queries, find product information, or contact your local sales representative.

www.roche-applied-science.com/pack-insert/1603558a.pdf

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