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DIG Easy Hyb Granules

Hybridization solution for nucleic acid blots with digoxigenin-labeled probes

Cat. No. 11 796 895 001

Version 06

Content version: October 2004

Granules for 6 x 100 ml

Store this product at 15 to 25°C!

1. Product overview

Contents

Bottles	Label	Contents
6	DIG Easy Hyb Granules	Granules for reconstitution

Product description

DIG Easy Hyb Granules are non-toxic and do 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 buffer 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 buffer is performed for 15-30 min at the appropriate hybridization temperature.

Hybridization

DIG Easy Hyb buffer 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	2 hours
Single-copy gene detection in human genomic blots	overnight
RNA:RNA hybridization	6 h - overnight
Oligonucleotide probes	1-6 hours

Storage/ stability

The unopened bottle is stable at +15 to +25 °C until the expiration date printed on the label.

We recommend to use up reconstituted DIG Easy Hyb buffer during 3 months.

Storage of DIG-labeled probes in DIG Easy Hyb

Probes labeled with digoxigenin can be stored in DIG Easy Hyb buffer at -15 to -20°C and be re-used several times, after denaturing at 65°C prior to use.

2. Procedures and required materials

2.1 Before you begin

Preparation of working solution

Add carefully 64 ml autoclaved double distilled water in two portions to the plastic bottle, dissolve by stirring immediately for 5 min at 37°C.

Note: For applications with Northern blots, DIG Easy Hyb Granules must be solved under RNase-free conditions.

Additional equipment required

Hybridization can be performed in temperature resistant, sealable

- Hybridization bags*
- 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 and percent homology of probe to target 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 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 buffer is 20-25°C below the calculated T_m value.

Example

For hybridization of human 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 buffer (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 buffer.
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 buffer (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 single copy detection we recommend o/n incubation.

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 buffer (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 buffer.
3	Denature DIG-labeled RNA probe (100 ng/ml hybridization solution) by boiling for 5 min and rapidly cooling on ice-water. **Please note:** In the case of reuse of DIG labeled RNA probe in DIG Easy Hyb please denature at 65°C, do not boil!
4	Add to pre-heated DIG Easy Hyb buffer (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	IIn 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 buffer (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 buffer.
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 buffer (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. 11 467 140 001) 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 buffer (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 buffer.
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 buffer per 100 cm² of membrane.
4	Add to pre-heated DIG Easy Hyb buffer (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) • 20x 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 buffer 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 buffer. • 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 buffer, 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 se-veral times when freshly denatured.

2.7 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 2x 5 min in ample 2x SSC; SDS 0.1% at 15-25°C.
2	Wash 2x 15 min in 0.5x 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 autoclaved double distilled water.
3	Wash for 2 x 20 min in 0.2 N NaOH, SDS, 0.1% (w/v) at 37°C under constant agitation.
4	Equilibrate briefly in 2x SSC.
5	Prehybridize and incubate with second probe.

3. References

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

Please refer to our website for the following informations:
2 DIG Special Interest Site: http://www.roche-applied-science.com/DIG/
3 DIG Product Selection Guide
4 DIG Application Manual for Filter Hybridization
5 Non-radioactive In situ Hybridization Manual
6 Lab FAQS

3.1 Changes to previous version

- Regulatory disclaimer updated
- Editorial changes

4. Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage http://www.roche-applied-science.com and our Special Interest Sites including:

- DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection http://www.roche-applied-science.com/DIG/.

Kits

Product	Pack Size	Cat. No
DIG DNA Labeling Kit	40 labeling reactions	11 175 033 910
DIG RNA Labeling Kit (SP6/T7)	2 x 10 reactions	11 277 073 910
DIG Oligonucleotide 3'-End Labeling Kit , 2nd Generation	25 reactions	03 353 575 910
DIG Oligonucleotide Tailing Kit, 2nd Generation	25 reactions	03 353 583 910
DIG DNA Labeling and Detection Kit	25 labeling reactions and 50 blots (10 x 10 cm)	11 093 657 910
DIG Nucleic Acid Detection Kit	40 blots (10 x 10 cm)	11 175 041 910
DIG Luminescent Detection Kit for Nucleic Acids	50 blots (10 x 10 cm)	11 363 514 910

Single reagents

Product	Pack Size	Cat. No.
Nylon membranes, positively charged	10 sheets (20 x 30 cm) 20 sheets (10 x 15 cm) 1 roll (0.3 x 3 m)	11 209 272 001 11 209 299 001 11 417 240 001
Nylon Membranes for Colony and Plaque Hybridization	50 filters (∅ 82 mm) 50 filters (∅ 132 mm)	11 699 075 001 11 699 083 001
DNA, MB-grade	500 mg (50 ml)	11 467 140 001
Hybridization bags	50 bags	11 666 649 001

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To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

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