For life science research only.
Not for use in diagnostic procedures.

DIG Easy Hyb

Version: 14
Content Version: December 2020

Hybridization solution for nucleic acid blots with digoxigenin-labeled probes

Cat. No. 11 603 558 001 500 ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG Easy Hyb	Ready-to-use solution, DNase and RNase free	1 bottle, 500 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	DIG Easy Hyb	Store at +2 to +8°C. ⚠ ***Aliquot the solution and store at +2 to +8°C.*** ⚠ ***If precipitates are visible in the solution, warm the bottle at +37°C for 10 minutes. Do not warm up more than 10 times.***

1.3. Additional Equipment and Reagent required

For Hybridizations

- Temperature resistant and sealable Hybridization Bags*, plastic or glass boxes, petri dishes, or roller bottles

For Post Hybridization Washes, Stripping, and Rehybridization

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

For Immunological Detection

- DIG Luminescent Detection Kit* or
- DIG Wash and Block Buffer Set*

1.4. Application

DIG Easy Hyb can be used for all types of nucleic acid blot hybridizations. It is especially designed for use with digoxigenin (DIG-labeled) nucleic acid probes to targets bound to nylon membranes.

Product Description

DIG Easy Hyb is a nontoxic, ready-to-use hybridization buffer. DIG Easy Hyb does not contain formamide, but facilitates lowering of hybridization temperature comparable to buffers that contain 50% formamide.

1.5. Preparation Time

Assay Time

Prehybridization

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

Hybridization

DIG Easy Hyb can drastically reduce hybridization times to only 1 to 6 hours, depending on the type of hybridization. Only for high sensitivity requirements is overnight (12 to 16 hours) hybridization 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 genomic blots	Overnight
RNA:RNA hybridization	6 hours to overnight
Oligonucleotide probes	1 – 6 hours

2. How to Use this Product

2.1. Before you Begin

General Considerations

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. The probes can be reused several times after denaturing at +65°C prior to use.
⚠ ***Do not boil.***

Hybridization Temperatures

Hybridization Type	Hybridization Temperature
DNA:DNA	The appropriate hybridization temperature is calculated based on the GC content and percent homology of probe to target and according to the following equation: Tm = 49.82 + 0.41 (%G + C) − (600/l) ⓘ *l = length of hybrid in base pairs.* $T_{opt.}$ = Tm − (+20 to +25°C) ⓘ *The given numbers of the equation were calculated according to a standard equation for hybridization solutions containing 50% formamide.* The actual hybridization temperature $T_{opt.}$ for hybridization with DIG Easy Hyb is +20 to +25°C below the calculated Tm value. **Example** For hybridization of human genomic DNA with a 100% homologous probe, use +37 to +42°C, depending on the GC content of the probe.
RNA:RNA	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.
DNA:RNA	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.
Using DIG-Labeled Oligonucleotide Probes	The hybridization temperature is calculated as follows: Calculate Tm 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 Tm. **Multiple Locus Fingerprinting Probes** For multiple locus fingerprinting probes, we recommend 2 to 4 hours hybridization time. Nonspecific competitor DNA such as DNA, MB-grade* from fish sperm should be added at a concentration of 50 µg/ml.
Plaque and Colony	The appropriate hybridization temperature is calculated according to GC content and percent homology of probe to target DNA, and according to the following equation: Tm = 49.82 + 0.41 (%G + C) − (600/l) ⓘ *l = length of hybrid in base pairs.* $T_{opt.}$ = Tm − (+20 to +25°C) The actual hybridization temperature $T_{opt.}$ with DIG Easy Hyb is +20 to +25°C below Tm.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

DNA:DNA Hybridization

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

1. Preheat the appropriate volume of DIG Easy Hyb (approximately 20 ml/100 cm^2) to hybridization temperature.

2. Incubate the blot for 15 to 30 minutes with gentle agitation.
   i The membrane should be completely immersed and covered with DIG Easy Hyb.

3. Denature the DIG-labeled DNA probe (5 to 25 ng/ml hybridization solution) by boiling for 5 minutes and rapidly cooling in ice water.

4. Add denatured probe to preheated DIG Easy Hyb (at least 3.5 ml/100 cm^2 membrane).
   – Mix well avoiding foam, as bubbles may lead to background.

5. Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane.
   i Do not add concentrated probe directly to membrane to avoid localized background.

6. Incubate with gentle agitation for at least 6 hours at hybridization temperature.

   i For single-copy detection, we recommend overnight incubation.

RNA:RNA Hybridization

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

1. Preheat the appropriate volume of DIG Easy Hyb (approximately 20 ml/100 cm^2) to hybridization temperature.

2. Incubate the blot for 30 minutes with gentle agitation.
   i The membrane should be completely immersed and covered with DIG Easy Hyb.

3. Denature the DIG-labeled RNA probe (100 ng/ml hybridization solution) by boiling for 5 minutes and rapidly cooling in ice water.
   ⚠ ***If reusing the DIG-labeled RNA probe in DIG Easy Hyb, denature the probe at +65°C. Do not boil.***

4. Add denatured probe to preheated DIG Easy Hyb (at least 3.5 ml/100 cm^2 membrane).
   – Mix well avoiding foam, as bubbles may lead to background.

5. Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane.
   Do not add concentrated probe directly to membrane to avoid localized background.

6. Incubate with gentle agitation for at least 6 hours at hybridization temperature.

   For detection of rare mRNAs, we recommend 16 hour incubation.

DNA:RNA Hybridization

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

1. Preheat the appropriate volume of DIG Easy Hyb (approximately 20 ml/100 cm^2) to hybridization temperature.

2. Incubate the blot for 30 minutes with gentle agitation.
   The membrane should be completely immersed and covered with DIG Easy Hyb.

3. Denature the DIG-labeled probes (5 to 25 ng/ml in hybridization solution for DNA probes, 100 ng/ml hybridization solution for RNA probes) by boiling for 10 minutes and rapidly cooling in ice water.

4. Add denatured probes to preheated DIG Easy Hyb (at least 3.5 ml/100 cm^2 membrane).
   – Mix well avoiding foam, as bubbles may lead to background.

5. Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane.
   Do not add concentrated probe directly to membrane to avoid localized background.

6. Incubate with gentle agitation for at least 6 hours at hybridization temperature.

   For detection of rare mRNAs, we recommend 16 hour incubation.

Hybridization with DIG-Labeled Oligonucleotide Probes

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

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

1. Preheat the appropriate volume of DIG Easy Hyb (approximately 20 ml/100 cm^2) to hybridization temperature.

2. Incubate the blot for 30 minutes with gentle agitation.
   The membrane should be completely immersed and covered with DIG Easy Hyb.

3. Hybridize with 0.1 to 2 pmol tailed oligonucleotide/ml of hybridization solution or 1 to 10 pmol of end-labeled oligonucleotide.
   – Use at least 3.5 ml DIG Easy Hyb per 100 cm^2 of membrane.

4. Add to preheated DIG Easy Hyb (at least 3.5 ml/100 cm^2 membrane).
   – Mix well avoiding foam, as bubbles may lead to background.

5. Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane.
   Do not add concentrated probe directly to membrane to avoid localized background.

6. Incubate with gentle agitation for 1 to 6 hours at hybridization temperature.

   For detection of rare mRNAs, we recommend 16 hour incubation.

Plaque and Colony Hybridization

The following volumes are calculated when using a 275 ml volume roller bottle. The hybridization temperature is given for a 100% homologous probe with 50% GC content.

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

⚠ ***Be sure that the membranes do not stick to each other and are sufficiently covered with hybridization solution.***

1. Place 3 membrane discs (82 mm diameter) in a roller bottle and add 60 ml DIG Easy Hyb.
   – Prehybridize for 1 hour at +42°C in a hybridization oven for roller bottles.

2. Denature the labeled probe (25 ng/ml hybridization solution) by boiling for 5 minutes at +95 to +100°C and rapidly place on ice.

3. Add denatured probe (5 to 25 ng/ml) to the DIG Easy Hyb, preheated to hybridization temperature.

4. Pour off prehybridization solution and add 6 ml of the probe/DIG Easy Hyb mixture.

5. Incubate for 2 hours at +42°C.

ⓘ *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.*

Immunogical Detection

The procedure for the immunological detection of DIG-labeled nucleic acids is described in the Instructions for Use of the DIG Luminescent Detection Kit* or the DIG Wash and Block Buffer Set*.

Post Hybridization Washes, Stripping, and Rehybridization

Post Hybridization Washes

1. Wash 2 × 5 minutes in sufficient amounts of 2x SSC, 0.1% SDS at +15 to +25°C.

2. Wash 2 × 15 minutes in 0.1x SSC, 0.1% SDS at +68°C under constant agitation.

Stripping and Rehybridization

ⓘ *When stripping and rehybridization of blots is planned, do not allow the membrane to dry out at any time.*

⚠ ***Always work under a fume hood.***

1. Preheat dimethylformamide (DMF) in a water bath to +50 to +60°C and incubate the membrane until the color (NBT/BCIP) is washed off.
   ⓘ *Step 1 only applies to color detection.*
   ⚠ ***DMF is volatile and can be ignited above +67°C.***

2. Rinse membrane briefly in PCR-grade water.

3. Wash for 2 × 20 minutes in 0.2 M NaOH, 0.1% (w/v) SDS at +37°C under constant agitation.

4. Equilibrate briefly in 2x SSC.

5. Prehybridize and incubate with second probe.

3. Supplementary Information

3.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

3.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Consumables		
Hybridization Bags	50 bags, 25 cm x 23 cm	11 666 649 001
Reagents, kits		
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
DNA, MB-grade	500 mg, 50 ml, 10 mg/ml	11 467 140 001
Nylon Membranes for Colony and Plaque Hybridization	50 discs, 82 mm diameter	11 699 075 001
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001

3.4. Trademarks

DIG EASY HYB is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

3.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

3.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

3.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

3.8. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

1220.11601369001 ©

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