

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



Actin RNA Probe, DIG-labeled

 **Version: 18**

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Cat. No. 11 498 045 910 2 µg

Store the product at –15 to –25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Actin RNA Probe, digoxigenin-labeled	10 µg/ml in aqueous solution.  Supplied in autoclaved, DEPC-treated water.	1 vial, 2 µg

1.2. Storage and Stability


Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Actin RNA probe, digoxigenin-labeled	Store at –15 to –25°C.

1.3. Additional Equipment and Reagent required

For RNA:RNA hybridization with DIG Easy Hyb buffer

- DIG Easy Hyb* or
 - DIG Easy Hyb Granules*
 - Hybridization bags*, or
 - Plastic or glass boxes, petri dishes or roller bottles
 - Nylon Membranes, positively charged*
-  Do not use open trays when working with DIG Easy Hyb buffer.

For detection of DIG-labeled probes

- DIG Luminescent Detection Kit*
- DIG Nucleic Acid Detection Kit*

1.4. Application

Use the Actin RNA probe, digoxigenin-labeled for evaluating the quality and quantity of various RNA species and for the following applications:

- *In situ* hybridization, for example, as a control in mRNA detection.
- Quality control in the construction of cDNA libraries.
- Northern blot analysis to evaluate RNA from various human cell lines and tissue samples.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Precautions

- Avoid contamination of the Actin RNA probe with RNase during use.
- Always keep the Actin RNA probe, digoxigenin-labeled preparation on ice.

2.2. Protocols

Simultaneous hybridization with different DIG-labeled RNA probes

The Actin RNA probe, digoxigenin-labeled can be hybridized simultaneously with a different digoxigenin-labeled RNA probe, so that bands for the respective RNAs can be observed in the same lane and the expression level of the target mRNA can be evaluated. Such evaluation requires that the molecular weights of the actin (approximately 1.8 kb) and target mRNA differ enough to give well resolved bands in northern blot analysis.

Separate hybridizations

If separate hybridizations are to be carried out for the actin and target probes, refer to the following steps.

- 1 Detection of target mRNA.

- 2 Equilibration of the blot for 30 minutes at +15 to +25°C in prehybridization buffer.

- 3 Prehybridization at the hybridization temperature in fresh prehybridization solution.

- 4 Hybridization with the labeled actin probe.

RNA:RNA hybridizations with DIG Easy Hyb buffer

When using DIG Easy Hyb buffer for RNA:RNA hybridizations, consider the following:

Parameter	Consideration
Probe concentration	Standard hybridization concentration of the Actin RNA probe, digoxigenin-labeled is 100 ng/ml. <i>i</i> The Actin RNA probe, digoxigenin-labeled can be hybridized according to the conditions generally used with DIG RNA probes at a concentration of 50 to 200 ng/ml; higher concentrations are suitable when the amount of RNA on the blot is low or when a faster detection is desired.
Prehybridization	Prehybridization with DIG Easy Hyb* buffer is performed for 15 to 30 minutes at the appropriate hybridization temperature.
Hybridization temperature	Use a hybridization temperature of +68°C for RNA:RNA hybridization. <i>i</i> The actual hybridization temperature may need to be adjusted depending on the GC content and homology of probe to target.

RNA:RNA hybridization with digoxigenin-labeled RNA probe

- 1 Pre-heat appropriate volume of DIG Easy Hyb buffer (approximately 20 ml/100 cm²) to +68°C.

- 2 Incubate the membrane for 30 minutes with gentle agitation.
 ⚠ **Completely cover the membrane with DIG Easy Hyb buffer.**

- 3 Denature DIG-labeled RNA probe (100 ng/ml hybridization solution) by boiling for 5 minutes 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; avoid foaming 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 avoid localized background.**

- 6 Incubate with gentle agitation for at least 6 hours at +68°C.

Detection of DIG-labeled probes

Two methods can be used for detection:

Method	Kit
Chemiluminescent	DIG Luminescent Detection Kit*
Colorimetric	DIG Nucleic Acid Detection Kit*

2.3. Parameters

Sensitivity

- Agarose gel electrophoresis under denaturing conditions, and subsequent northern blot analysis reveal a defined band of 588 bases.
- Dot blot analysis with the DIG Nucleic Acid Detection Kit* allows the detection of 0.3 pg digoxigenin-labeled actin RNA probe.

3. Additional Information on this Product

3.1. Test Principle

Mammalian genomes carry a variety of genes for the various isoforms of actin, of which some are expressed in a tissue-specific manner and others exist as pseudogenes. Actins are highly conserved, and are found in nearly all eukaryotic cells.

Preparation

The antisense RNA probe (human β -actin) was *in vitro* transcribed in the presence of digoxigenin-UTP. The transcript has a length of 588 bases. 550 bases are complementary to the 5'-region of human β -actin mRNA (nucleotides 69 to 618, EMBL: HSAC07). The additional 25 bases at the 5' end, and the 13 additional bases at the 3' end of the transcript are specific for the promoter/polylinker region of the transcription vector.

4. Supplementary Information

4.1. 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.**

   etc. Stages in a process that usually occur in the order listed.

   etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Consumables		
Hybridization Bags	50 bags, 25 cm x 23 cm	11 666 649 001
Reagents, kits		
DIG Easy Hyb Granules	6 bottles, Granules for 6 x 100 ml	11 796 895 001
DIG Easy Hyb	500 ml	11 603 558 001
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
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm ²	11 363 514 910

4. Supplementary Information

4.4. Trademarks

DIG EASY HYB is a trademark of Roche.

All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

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

4.8. 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.

