

Actin RNA probe, digoxigenin-labeled

Cat. No. 11 498 045 910

2 µg

Version Nov. 2008

Store at -15 to -25°C

1. Product overview

Contents	[10 µg/ml] in aqueous solution. Note: The digoxigenin-labeled actin RNA probe is supplied as a sterile solution and is free of contaminating RNase activity. Care should be taken to avoid contamination of the probe with RNase during use.
Product description	The antisense RNA probe (human β-actin) was <i>in vitro</i> 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) (1). 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.
Background	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 nearly found in all Eukaryotic cells (2).
Application	The actin RNA probe is especially useful for evaluating the quality and quantity of various RNA species and can be used for <ul style="list-style-type: none">• <i>in situ</i> hybridization (for example, as a control in mRNA detection)• for quality control in the construction of cDNA libraries (3).• Northern blot analysis to evaluate RNA from various human cell lines and tissue samples
Storage/ Stability	The unopened vial is stable at -15 to -25°C through the expiration date printed on the label. Note: The digoxigenin-labeled actin RNA probe is delivered on dry ice. During use, the preparation should be maintained on ice.
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.
Simultaneous hybridization with different DIG-labeled RNA probes	The digoxigenin labeled actin RNA probe 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 (approx. 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, please refer to the following table.

Stage	Description
1	Detection of target mRNA.
2	Equilibration of the blot for 30 min 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.

2. RNA : RNA hybridization with DIG Easy Hyb

Additional material required

- DIG Easy Hyb* or
- DIG Easy Hyb Granules*

Additional equipment required

Hybridization can be performed in temperature resistant:

- Hybridization bags*
- plastic or glass boxes
- petri dishes
- roller bottles

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

Probe concentration

- Standard hybridization concentration of the digoxigenin-labeled actin RNA probe: 100 ng/ml,
Note: The digoxigenin-labeled actin RNA probe 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 is performed for 15-30 min at the appropriate hybridization temperature.

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 with digoxigenin-labeled RNA probe is described.

Step	Action
1	Pre-heat appropriate volume of DIG Easy Hyb (approx. 20 ml/ 100 cm ²) to 68°C.
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 cm ² 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 68°C.

Detection of DIG labeled probes

Two methods can be used for detection:

- the chemiluminescent one with the DIG Luminescent Detection Kit*
- and the colorimetric one with DIG Nucleic Acid Detection Kit*

3. Appendix

3.1 References

- 1 Ponte, P. et al. (1984) *Nucleic Acids Res.* **12**, 1607-1696.
- 2 Rubenstein, P. A. (1990) *BioEssays* **12**, 309-315.
- 3 Hagen, F. S. et al. (1988) *BioTechniques* **6**, 340-344

Please refer to our website for the following informations:

- 4 DIG Product Selection Guide
- 5 DIG Application Manual for Filter Hybridization
- 6 Non-radioactive In situ Hybridization Manual
- 7 Lab FAQs

Changes to Previous Version

- New Disclaimer
- Ordering Information updated

3.3 Ordering Information

For a complete overview of related products, please visit and bookmark 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 RNA Labeling Kit (SP6/T7)	2 × 10 labeling reactions	11 175 025 910
DIG Nucleic Acid Detection Kit	40 blots (10 × 10 cm)	11 175 041 910
DIG Northern Starter Kit	10 labeling reactions and detection of 10 × 10 cm ² membrane	12 039 672 910
DIG Luminescent Detection Kit	1 kit (50 blots)	11 363 514 910

Single reagents

Product	Pack Size	Cat. No.
DIG RNA Labeling Mix	40 µl, 20 reactions	11 277 073 910
Digoxigenin-11-UTP	250 nmol (25 µl)	11 209 256 910
DIG-labeled control RNA	50 ml	11 585 746 910
DIG Easy Hyb	500 ml	11 603 558 001
DIG Wash and Block Buffer Set	30 blots (10 × 10 cm ²)	11 585 762 001
RNA Molecular Weight Marker, digoxigenin-labeled: RNA MWM I RNA MWM II RNA MWM III	4 µg (200 µl) 2 µg (200 µl) 2 µg (200 µl)	11 526 529 910 11 526 537 910 11 373 099 910
Hybridization bags	50 bags	11 666 649 001
Nylon Membrane, positively charged	10 sheets (20 × 30 cm) 20 sheets (10 × 15 cm) 1 roll (0.3 × 3 m roll)	11 209 272 001 11 209 299 001 11 417 240 001

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