

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



DIG-labeled Control RNA

 **Version: 11**

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Cat. No. 11 585 746 910 50 µl
100 µg/ml DIG-labeled RNA

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	DIG-labeled Control RNA, 5 µg/50 µl	<ul style="list-style-type: none"> Contains 50 µl digoxigenin-labeled anti-sense neo-RNA solution. Solution contains approximately 5 µg of DIG-labeled neo-RNA (760 bp length) and 0.5 µg pSPT18-neo template DNA (fragments of 798 and 3,281 bp). 	1 vial, 50 µl

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-labeled Control RNA	Store at –15 to –25°C.

1.3. Additional Equipment and Reagent required

RNA dilution buffer: Mix DEPC-treated double-distilled water, 20x SSC, and formaldehyde in a ratio of 5:3:2.

1.4. Application

The amount of DIG-labeled RNA can be estimated in comparison to the DIG-labeled Control RNA according to the procedure described in the DIG Nucleic Acid Detection Kit* and DIG RNA Labeling Kit (SP6/T7)*.

2. How to Use this Product

2.1. Protocols

Semi-Quantitative Determination of Labeling Efficiency

Determination of the yield of DIG-labeled RNA is most important for optimal and reproducible hybridization results. Too high of a probe concentration in the hybridization step causes background, while too low of a concentration leads to weak signals.

Dilution Series

To prepare the dilution series shown below, labeled probes and the DIG-labeled Control RNA must be diluted to 10 ng/μl, according to the expected yield of synthesized nucleic acid. The yield depends on the starting amount of template and incubation time.

Tube	RNA [μl]	From Tube No.	RNA Dilution Buffer [μl]	Dilution	Final Concentration
1	–	Diluted original	–	–	10 ng/μl
2	2	1	18	1:10	1 ng/μl
3	5	2	495	1:100	10 pg/μl
4	15	3	35	1:3.3	3 pg/μl
5	5	3	45	1:10	1 pg/μl
6	5	4	45	1:10	0.3 pg/μl
7	5	5	45	1:10	0.1 pg/μl
8	5	6	45	1:10	0.03 pg/μl
9	5	7	45	1:10	0.01 pg/μl
10	0	–	50	–	0

Probe Quantification

Spot a concentration series of the labeled probe RNA and the DIG-labeled Control RNA dilutions onto a positively charged nylon membrane and follow the procedure in the DIG Nucleic Acid Detection Kit.

2.2. Parameters

Sensitivity in Southern and Northern Blots

Using 100 ng/ml of the DIG-labeled Control RNA as a probe according to the standard protocol of the DIG Nucleic Acid Detection Kit, 0.1 pg homologous DNA or 0.1 pg homologous RNA can be detected on a dot blot after a 16 hour color development, or <30 minutes exposure to an X-ray film when using the chemiluminescent substrate CSPD*.

3. Additional Information on this Product

3.1. Test Principle

The preferred method for semi-quantification of labeled probes is the direct detection method in comparison to the Control RNA.

- ① A series of dilutions of DIG-labeled RNA is applied to a small strip of Nylon Membrane, positively charged*.
 - Part of the Nylon Membrane is preloaded with defined dilutions of Control RNA, used as standards.

 - ② The Nylon Membrane is subjected to immunological detection with Anti-Digoxigenin-AP* and a chemiluminescent substrate such as CDP-*Star** or CSPD*; a color detection with NBT/BCIP* is also possible.
 - The intensities of the dilution series of DIG-labeled RNA and Control RNA are compared by exposure to X-ray film or Lumi-Film*.
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3.2. Quality Control

0.1 pg of the DIG-labeled Control RNA can be visualized in a spot test with direct color detection.

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	
i	<i>Information Note: Additional information about the current topic or procedure.</i>
⚠	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.
1 2 3 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.
Reagents, kits		
NBT/BCIP Ready-to-Use Tablets	20 tablets	11 697 471 001
NBT/BCIP Stock Solution	8 ml	11 681 451 001
DIG RNA Labeling Kit (SP6/T7)	1 kit, 2 x 10 labeling reactions	11 175 025 910
Lumi-Film Chemiluminescent Detection Film	100 films, 7.1 x 9.4 inches, 18 x 24 cm, <i>Not available in US</i>	11 666 916 001
	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910
CDP- <i>Star</i> , ready-to-use	2 x 50 ml	12 041 677 001
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
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910

4.4. Trademarks

All 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.

