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

DIG RNA Labeling Mix

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RNA labeling with digoxigenin-UTP by *in vitro* transcription with SP6, T7, and T3 RNA polymerase for 20 transcriptions

Cat. No. 11 277 073 910 40 µl
20 transcriptions

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG RNA Labeling Mix, 10x conc.	▪ For 20 transcriptions. ▪ Contains 10 mM ATP, 10 mM CTP, 10 mM GTP, 6.5 mM UTP, and 3.5 mM DIG-11-UTP, pH 7.5 (20°C).	1 vial, 40 µl

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	DIG RNA Labeling Mix, 10x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing. To avoid contamination, aliquot the DIG RNA Labeling Mix into 2 to 3 vials.***

1.3. Additional Equipment and Reagent required

For Standard Labeling Assay

- SP6 RNA Polymerase* or
- T7 RNA Polymerase* or
- T3 RNA Polymerase* or
- DNase I, RNase-free* (optional)

Transcription buffer, 10x concentrated, is supplied with the RNA polymerases: 400 mM Tris-HCl, pH 8.0 (20°C), 60 mM $MgCl_2$, 100 mM dithiotreitol (DTT), and 20 mM spermidine.

For Ethanol Precipitation of RNA Transcripts (Optional)

- 4 M LiCl, RNase-free
- Prechilled (−15 to −25°C) ethanol
- Prechilled (−15 to −25°C) ethanol, 70% (v/v)
- Sterile, RNase-free double-distilled water

1.4. Application

DIG-labeled RNA is used for hybridization to:

- Northern blots
- Southern blots
- Plaque or colony lifts, or
- RNase protection experiments
- Chromosomes, cells, and tissue sections *in situ.*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Linearized Plasmid DNA

The DNA to be transcribed is cloned into the polylinker site of an appropriate transcription vector which contains, adjacent to the polylinker, a promoter for SP6, T7, or T3 RNA polymerase (Smith, G.R., and Summers, M.D., 1990). For the synthesis of run-off transcripts, the plasmid is linearized by a restriction enzyme. Restriction enzymes creating 5′ overhangs should be used; 3′ overhangs should be avoided. The linearized template DNA should be purified by phenol-chloroform extraction and ethanol precipitation to avoid RNase contamination. For run-around transcription, circular plasmid DNA is used.

PCR Product

PCR fragments which contain RNA polymerase promoter sequences can also be used as templates for transcription. Purification of the correct PCR fragment by gel electrophoresis prior to transcription is recommended.

General Considerations

Analysis of Labeled RNA

Quality and quantity of the transcript can be analyzed by non-denaturing agarose gel electrophoresis and ethidium bromide staining. The signal from the RNA band should be approximately 10 fold stronger than that from the DNA. The size and the amount of the transcript can be estimated by comparison to known RNAs.

Determination of Labeling Efficiency

The efficiency of the DIG-labeling reaction should be determined in a spot assay: Prepare dilutions (10 pg/µl to 0.01 pg/µl) of the DIG-labeled transcript in sterile, RNase-free double-distilled water and spot 1 µl of each dilution onto a Nylon Membrane, positively charged*. After fixation of the RNA to the membrane by UV-treatment or baking, the detection is performed as described in the DIG Luminescent Detection Kit*. Semi-quantitative determination is done by comparison with dilution of a standard DIG-labeled control RNA of known concentration (DIG-labeled Control RNA*).

Storage of Labeled RNA

For long-term storage, labeled RNA should be precipitated with ethanol and also be stored under ethanol at −15 to −25°C or −60°C or below.

Hybridization with Labeled RNA

Check the labeling efficiency in a spot assay (see **Determination of Labeling Efficiency**) and then apply either 20 ng (for abundant, for example, housekeeping gene transcript detection) or up to 100 ng (for rare transcript detection) of the DIG-labeled RNA per ml hybridization solution. We recommend the use of DIG Easy Hyb* buffer for strong signals and background-free results.

Detection of DIG-Labeled RNA Probes

After hybridization to nucleic acid targets bound to a nylon membrane, the DIG label is detected by an immunoassay with Anti-DIG-AP conjugate and the chemiluminescent substrates CSPD* or CDP-*Star**. Detailed detection protocols are available in the Instructions for Use of the substrates, the DIG Luminescent Detection Kit*, and others (available on request).

DNase Treatment

When the DIG-labeled RNA is used for hybridization to northern or Southern blots, plaque or colony lifts, DNase treatment is not required, as the amount of DIG-labeled RNA transcript is far in excess of the template DNA.

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

Standard Labeling Assay

⚠ ***Always work under RNase-free conditions.***

1. Add the following to a microcentrifuge tube on ice:

Reagent	Volume [µl]
1 µg linearized plasmid DNA or appropriate amount of PCR product (100 to 200 ng)	X
DIG RNA Labeling Mix, 10x	2
Transcription buffer, 10x	2
Add sterile RNase-free double-distilled water to a final volume of 18 µl	X
RNA polymerase (SP6, T7, or T3), 20 U/µl	2
Total Volume	**20**

2. Mix and centrifuge briefly.
   – Incubate for 2 hours at 37°C.

3. Optional: Add 2 µl DNase I, RNase-free to remove template DNA.
   – Incubate for 15 minutes at 37°C.
   Only required for RNase-protection experiments.

4. Stop the reaction by adding 2 µl of 0.2 M EDTA (pH 8.0).

Ethanol Precipitation of RNA Transcripts (Optional)

For ethanol precipitation of the 22 µl standard assay:

1. Add 2.5 µl (0.1 volume) 4 M LiCl and 75 µl (2.5 volumes) prechilled (−15 to −25°C) ethanol to the standard reaction and mix well.
2. Let sit at least 30 minutes at −60°C or below, or 2 hours at −15 to −25°C.
3. Centrifuge at 13,000 × *g* for 15 minutes at +2 to +8°C.
4. Decant the ethanol and wash the pellet carefully with 50 µl cold (−15 to −25°C) ethanol, 70% (v/v).
5. Centrifuge at 13 000 × *g* for 5 minutes.
6. Decant the ethanol and dry the pellet briefly under vacuum.
7. Dissolve the pellet in 50 µl sterile, RNase-free double-distilled water or TE buffer.
8. Add 1 µl Protector RNase Inhibitor* (optional) and use immediately or store at −60°C or below in aliquots.

3. Results

Labeling Efficiency

In the standard reaction, approximately 10 µg full length DIG-labeled RNA is synthesized from 1 µg linearized plasmid DNA with an insert of approximately 1 kb in 2 hours. Larger amounts of DIG-labeled RNA can be obtained by scaling up the reaction components. The amount of synthesized labeled RNA depends on the amount, size (site of linearization), and purity of the template DNA.

Longer incubations do not increase the yield of labeled RNA.

4. Additional Information on this Product

4.1. Test Principle

DIG-labeled, single-stranded RNA probes of defined length are generated by *in vitro* transcription. DIG-11-UTP is incorporated by SP6, T7, and T3 RNA polymerases at approximately every 20 to 25th nucleotide of the transcript under the standard conditions (Feinberg, A.P., and Vogelstein, B., 1983; Southern, E.M., 1975). The DIG RNA Labeling Mix is specifically designed for the use with SP6, T7, and T3 RNA Polymerases from Roche, which are supplied with an optimized transcription buffer.

4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

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

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
T3 RNA Polymerase	1,000 U, ≥ 20 U/µl	11 031 163 001
	5,000 U, ≥ 20 U/µl	11 031 171 001
DIG RNA Labeling Kit (SP6/T7)	1 kit, 2 x 10 labeling reactions	11 175 025 910
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
CDP-*Star*, ready-to-use	2 x 50 ml	12 041 677 001
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
DIG Easy Hyb	500 ml	11 603 558 001
SP6 RNA Polymerase	1,000 U, > 20 U/µl	10 810 274 001
	5,000 U, > 20 U/µl	11 487 671 001
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-labeled Control RNA	50 µl, 100 µg/ml DIG-labeled RNA	11 585 746 910
T7 RNA Polymerase	1,000 U, ≥ 20 U/µl	10 881 767 001
	5,000 U, ≥ 20 U/µl	10 881 775 001
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910
Protector RNase Inhibitor	2,000 U, 40 U/µl	03 335 399 001
	10,000 U, 5 x 2,000 U	03 335 402 001

5.4. Trademarks

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

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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.

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