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Not for use in diagnostic procedures.

DIG Oligonucleotide 3’-End Labeling Kit, 2nd Generation

Version: 09
Content Version: November 2023

For labeling of oligonucleotides with digoxigenin-ddUTP.

Cat. No. 03 353 575 910 1 kit
25 labeling reactions of 100 pmol of oligonucleotide corresponding to 1 µg of a 30-mer oligonucleotide, each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Reaction Buffer, 5x conc.	1 M potassium cacodylate, 0.125 M Tris-HCl, 1.25 mg/mL bovine serum albumin, pH 6.6 (+25°C). ⚠ ***See section, Safety Information, Precautions for additional information on handling potassium cacodylate.***	1 vial, 100 µL
2	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Cobalt chloride Solution	25 mM $CoCl_2$ solution.	1 vial, 100 µL
3	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Digoxigenin-ddUTP Solution	1 mM DIG-11-ddUTP in double-distilled water.	1 vial, 25 µL
4	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Terminal Transferase	400 U/µL recombinant Terminal Transferase in 60 mM K-phosphate, pH 7.2 (+4°C), 150 mM KCl, 1 mM 2-mercaptoethanol, 0.1% Tween 20, 50% glycerol.	1 vial, 25 µL
5	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Control Oligonucleotide, unlabeled	20 pmol/µL Oligonucleotide: 30 mer, 5′-p TTG GGT AAC GCC AGG GTT TTC CCA GTC ACG OH-3′, homologous to the *lacZ*′ region in pUC and M13 plasmids in double-distilled water.	1 vial, 25 µL
6	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Control Oligonucleotide, DIG-ddUTP labeled	2.5 pmol/µL Oligonucleotide (sequence as in Vial 5), labeled under standard assay reaction conditions with DIG-ddUTP.	1 vial, 100 µL
7	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Control DNA, pUC 18	0.25 mg/mL pUC18 DNA (supercoiled) in 10 mM Tris-HCl, pH 7.6 (+25°C), 1 mM EDTA.	1 vial, 20 µL
8	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, Glycogen Solution	20 mg/mL Glycogen Solution in double-distilled water. ⓘ *The use of Glycogen as a precipitation aid is not described in this kit. It is not necessary to use precipitation to clean up the reaction after DIG labeling.*	1 vial, 50 µL
9	DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation, DNA Dilution Buffer	50 µg/mL fish sperm DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (+25°C).	1 vial, 1 mL

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Reaction Buffer, 5x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	Cobalt chloride Solution	
3	Digoxigenin-ddUTP Solution	
4	Terminal Transferase	
5	Control Oligonucleotide, unlabeled	
6	Control Oligonucleotide, DIG-ddUTP labeled	
7	Control DNA, pUC18	
8	Glycogen Solution	
9	DNA Dilution Buffer	

1.3. Additional Equipment and Reagent required

For oligonucleotide 3′-end labeling

See section, **Working Solution** *for additional information on how to prepare solutions.*

- Sterile, double-distilled water
- 0.2 M EDTA

For determination of labeling efficiency

- Whatman 3MM paper
- Nylon Membranes, positively charged*
- Anti-Digoxigenin-AP, Fab fragments*
- CDP-*Star*® or CSPD®, or the DIG Luminescent Detection Kit*
- DIG Wash and Block Buffer Set*
- TE buffer (optional)
- X-ray film or Lumi-Film*

For hybridization

- Nylon membranes, positively charged* or
- Nylon membranes, for Colony and Plaque Hybridization*
- Hybridization Bags* or
  – Temperature-resistant, sealable plastic or glass boxes, petri dishes, or roller bottles
- DIG Easy Hyb*
- Shaking water bath or hybridization oven

For post hybridization washes

- 2x SSC*,
- 0.1% SDS*
- 0.5x SSC*
- Shaker

For storage of filter (optional)

- 2x SSC* or
- Maleic acid buffer

For immunological detection

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

1.4. Application

The DIG Oligonucleotide 3′-End Labeling Kit can be used for:
- Dot and slot blotting
- Colony and plaque hybridization
- Southern blotting

1.5. Preparation Time

Assay Time

The entire procedure from oligonucleotide labeling to the hybridization and detection of the first visible signal is accomplished in <24 hours.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use oligonucleotides:
- Purified by HPLC or gel electrophoresis.
- Length from 14 to 100 nucleotides.

In one standard labeling reaction, up to 100 pmol (1 µg of a 30-mer) oligonucleotide can be applied.

Safety Information

Precautions

The Reaction Buffer (Vial 1) contains potassium cacodylate which is toxic.
- Always wear gloves when handling.
- Toxic by inhalation and if swallowed.
- Keep locked up and out of reach of children.
- When using, do not eat, drink, or smoke.
- After contact with skin, wash immediately with plenty of water.
- Collect the supernatants from the labeling reactions in a tightly closed, non-breakable container and indicate contents. Discard according to the applicable regulations for toxic waste.

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.

Working Solution

Solution	Composition	Storage and Stability	For use in...
Water	Autoclaved, double-distilled water	Store at + 2 to +8°C.	Dilution of oligonucleotides.
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0		Stopping the reaction.

2.2. Protocols

Oligonucleotide 3′-end labeling

The procedure described below, using 100 pmol 3′-OH ends (1 µg of a 30-mer oligonucleotide), will label almost all the oligonucleotides in the reaction vial.

⚠ ***Do not increase the amount of oligonucleotide in the labeling reaction. To label larger amounts of oligonucleotide, increase the reaction volume and all components proportionally, and increase incubation time to 1 hour.***

❶ Add 100 pmol oligonucleotide to a reaction vial and sterile, double-distilled water to a final volume of 10 µL.
– For the control reaction, add 5 µL Control Oligonucleotide, unlabeled (Vial 5) and 5 µL sterile, double-distilled water to a reaction vial.

❷ Place the reaction vial on ice and add the following:

Reagent	Volume [µL]	Final concentration
Reaction Buffer, 5x conc. (Vial 1)	4	1x
$CoCl_2$ Solution (Vial 2)	4	5 mM
DIG-ddUTP Solution (Vial 3)	1	0.05 mM
400 U Terminal Transferase (Vial 4)	1	20 U/µL
Final Volume	**10**	

– Mix and centrifuge briefly.
– Incubate at +37°C for 15 minutes, then place on ice.

❸ Stop the reaction by adding 2 µL 0.2 M EDTA, pH 8.0.
– Proceed to section, **Determination of labeling efficiency**.

ⓘ *It is not necessary to clean up the reaction prior to diluting the probe in hybridization buffer*

Determination of labeling efficiency

Determination of the yield of DIG-labeled oligonucleotides is critical for optimal and reproducible hybridization results. Quantification of labeled probes is performed via a direct detection method:

① A series of dilutions of DIG-labeled oligonucleotide is bound to a small strip of Nylon Membrane, positively charged*.
– Part of the nylon membrane is preloaded with known concentrations of DIG-labeled Control Oligonucleotide (Vial 6); these dilutions are used as standards.

② The labeled oligonucleotides on the nylon membrane are detected with Anti-digoxigenin-AP, Fab fragments* and the premixed stock solution of CSPD ready-to-use*.
– The chemiluminescent signals on the nylon membrane are analyzed with an appropriate imager or by exposure to X-ray film or Lumi-Film*; the signal intensities of the DIG-labeled oligonucleotide samples and the control oligonucleotide standards are compared to determine labeling yield.

Dilution series

Dilute a portion of your labeled oligonucleotide (100 pmol/22 µL) to a starting concentration of 2.5 pmol/µL. Using that initial dilution, prepare a dilution series of your oligonucleotide, as described in the following table. Prepare a similar dilution series with the labeled Control Oligonucleotide (Vial 6).

i *All dilutions can also be prepared with TE buffer.*

Tube No.	Oligo [µL]	From tube No.	Dilution Buffer (Vial 9) [µL]	Dilution	Final concentration [fmol/µL]
1	2	Original (2.5 pmol/µL)	48	1:25	100
2	3	1	7	1:3.3	30
3	2	1	18	1:10	10
4	2	2	18	1:10	3
5	2	3	18	1:10	1
6	–	–	20	–	0

Direct detection

1. To a strip of Nylon Membrane*, apply 1 µL spots of each dilution prepared above, for both the nucleotide dilutions and the control dilutions.

2. Fix the nucleic acid to the membrane by crosslinking with UV-light or baking for 30 minutes at +120°C.

3. Follow the chemiluminescent detection procedure described in the Instructions for Use of the DIG Luminescent Detection Kit* or the CSPD or CDP-*Star* substrates.

   i *Use reagent volumes appropriate to the size of your membrane strip.*

Analyzing the result

Compare the intensity of the spots from your labeling reaction to the intensity of the control spots. Use the relative intensities to calculate the amount of DIG-labeled oligonucleotide in your original sample.

Hybridization with DIG-labeled oligonucleotide probes

The hybridization and wash conditions listed below must be adjusted to suit the length and nucleotide composition of the oligonucleotides.

Hybridization temperature

The appropriate hybridization temperature is calculated with the following equation. Calculate the Tm of the oligonucleotide probe approximately:

[4°C × (each G or C) + 2°C × (each T or A)]

Perform prehybridization and hybridization at +10°C below the calculated Tm.

The following steps describe the hybridization procedure with DIG-labeled oligonucleotide probes.
⚠ ***Do not use open trays when working with DIG Easy Hyb buffer.***

1. Pre-heat appropriate volume of DIG Easy Hyb* buffer (approximately 20 mL/100 cm^2) to hybridization temperature.

2. Place the membrane into an appropriate container and pour the heated solution over the membrane.
   – Incubate for 30 minutes with gentle agitation.

   ⚠ ***Immerse the membrane completely in the DIG Easy Hyb buffer.***

3. Based on the calculations in section, **Determination of labeling efficiency**, obtain 1 to 10 pmol of your end-labeled probe.
   – Pre-heat ≥3.5 mL fresh DIG Easy Hyb per 100 cm^2 of membrane.

4. Add labeled probe from Step 3 to pre-heated DIG Easy Hyb buffer (≥3.5 mL/100 cm^2 membrane; from Step 3) and mix well.

   ⚠ ***Avoid foaming as bubbles may lead to background.***

5. Pour off prehybridization solution from Step 2 and immediately add probe/DIG Easy Hyb mixture to membrane.

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

   i *For detection of rare mRNAs, increase incubation time to 16 hours.*

Post hybridization washes

1. Wash 2 × 5 minutes in (2x SSC + 0.1% SDS) at +15 to +25°C.

2. Wash 2 × 15 minutes in (0.5x SSC + 0.1% SDS) at hybridization temperature.

   ⚠ ***Wash using constant agitation.***

IF...	THEN...
you want to continue,	use membrane directly for detection of hybridized oligonucleotide.
you want to stop,	air dry membrane and store for later detection.

Membrane handling

If membranes are to be stripped and reprobed, do not allow membrane to dry out; store membranes in 2x SSC or Maleic acid buffer.

Immunological detection of DIG-labeled oligonucleotides

After fixation and hybridization, detect DIG-labeled oligonucleotides with an antibody conjugated to the enzyme alkaline phosphatase which catalyzes a color or a chemiluminescent reaction.

- For color detection, use the DIG Nucleic Acid Detection Kit*.
- For chemiluminescent detection, use the DIG Luminescent Detection Kit*.
- Alternatively, especially for *in situ* applications, detect DIG-labeled hybrids with antibodies conjugated to different fluorochromes.

2.3. Parameters

Sensitivity

The nonradioactive DIG oligonucleotide 3′-end labeling and detection system allows the detection of 10 pg homologous DNA or RNA.

3. Additional Information on this Product

3.1. Test Principle

Labeling principle

- Terminal transferase is used to enzymatically label oligonucleotides at their 3′ ends by incorporation of a single digoxigenin-labeled dideoxyuridine-triphosphate (DIG-ddUTP).
- Oligonucleotides may also be labeled by addition of a longer nucleotide tail. A mixture of the deoxynucleotide triphosphates dATP and DIG-dUTP is used in a template-independent reaction to generate such tailed oligonucleotide probes (DIG Oligonucleotide Tailing Kit, 2nd generation*).

3.2. Quality Control

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

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.	
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.

4.2. Changes to previous version

Editorial changes.
Updated the section 1.1 “contents”: 0.5% Triton X-100 is replaced with 0.1% Tween 20.
Removed information related to the REACH Annex XIV.

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		
Buffers in a Box, Premixed SSC Buffer, 20x	4 L	11 666 681 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
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 of 10 cm x 10 cm	11 363 514 910
DIG Oligonucleotide Tailing Kit, 2nd Generation	1 kit, 25 tailing reactions of 100 pmol oligonucleotide corresponding to 1 µg of a 30-mer oligonucleotide	03 353 583 910
CSPD	1 mL	11 655 884 001
CDP-*Star*	1 mL	11 685 627 001
	2 x 1 mL	11 759 051 001
CSPD, ready-to-use	2 x 50 mL	11 755 633 001
CDP-*Star*, ready-to-use	2 x 50 mL	12 041 677 001
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µL	11 093 274 910
DIG Easy Hyb	500 ml	11 603 558 001
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

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:
Product Disclaimers.

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.

1123.03320570001 ©

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