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# **DIG Oligonucleotide Tailing Kit, 2nd Generation**

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For tailing of oligonucleotides with digoxigenin-dUTP using recombinant terminal transferase.

Cat. No. 03 353 583 910

1 kit
 25 tailing reactions of 100 pmol oligonucleotide corresponding to
 1 μg of a 30-mer oligonucleotide

Store the kit at −15 to −25°C.

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## 1. General Information

## **1.1. Contents**

Vial / Bottle	Label	Function / Description	Content
1	DIG Oligonucleotide Tailing 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).	1 vial, 100 µl
2	DIG Oligonucleotide Tailing Kit, 2nd Generation, Cobalt chloride hexahydrate	25 mM CoCl <sub>2</sub> solution.	1 vial, 100 µl
3	DIG Oligonucleotide Tailing Kit, 2nd Generation, Digoxigenin-dUTP solution	1 mM Digoxigenin-11-dUTP in double-distilled water.	1 vial, 25 µl
4	DIG Oligonucleotide Tailing Kit, 2nd Generation, dATP Solution	10 mM dATP in double-distilled water.	1 vial, 25 µl
5	DIG Oligonucleotide Tailing Kit, 2nd Generation, Terminal Transferase	400 U/µl recombinant Terminal Transferase in 60 mM K-phosphate (pH 7.2 at +4°C), 150 mM KCl, 1 mM 2-mercaptoethanol, 0.1% Tween 20, 50% glycerol.	1 vial, 25 µl
6	DIG Oligonucleotide Tailing 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 <i>lacZ</i> ' region in pUC and M13 plasmids in double-distilled water.	1 vial, 25 µl
7	DIG Oligonucleotide Tailing Kit, 2nd Generation, Control Oligonucleotide, DIG- dUTP/dATP	2.5 pmol/µl Oligonucleotide (sequence as in Vial 6), tailed under standard assay reaction conditions with DIG-dUTP/dATP in double-distilled water.	1 vial, 20 µl
8	DIG Oligonucleotide Tailing Kit, 2nd Generation, Control DNA, pUC18	0.25 mg/ml pUC18 DNA (supercoiled) in 10 mM Tris-HCl, pH 7.6 (+25°C), 1 mM EDTA.	1 vial, 20 µl
9	DIG Oligonucleotide Tailing Kit, 2nd Generation, Glycogen solution	<ul> <li>20 mg/ml Glycogen solution in double-distilled water.</li> <li><i>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.</i></li> </ul>	1 vial, 50 µl
10	DIG Oligonucleotide Tailing 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).	3 vials, 1 ml each
11	DIG Oligonucleotide Tailing Kit, 2nd Generation, Poly(A) Solution	<ul> <li>10 mg/ml Poly(A) Solution in double-distilled water.</li> <li><i>The amount of Poly(A) is sufficient for the preparation of 100 ml hybridization solution and should be used for hybridization with the Control Oligonucleotide.</i></li> </ul>	1 vial, 1 ml

## 1.2. Storage and Stability

### **Storage Conditions (Product)**

When stored at -15 to  $-25^{\circ}$ C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Reaction Buffer	Store at -15 to -25°C.
2	Cobalt chloride hexahydrate	Avoid repeated freezing and thawing.
3	Digoxigenin-dUTP solution	
4	dATP Solution	
5	Terminal Transferase	
6	Control Oligonucleotide, unlabeled	
7	Control Oligonucleotide, DIG-dUTP/dATP	
8	Control DNA, pUC18	
9	Glycogen solution	
10	DNA Dilution buffer	
11	Poly(A) Solution	

## **1.3. Additional Equipment and Reagent required**

#### For oligonucleotide tailing with DIG-11-dUTP/dATP

- Water bath
- Autoclaved, double-distilled water
- 0.2 M EDTA

#### For oligonucleotide tailing with nucleotides other than dATP

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

- Autoclaved, double-distilled water
- 0.2 M EDTA
- dATP\*, and/or dGTP\*, dCTP\*, dTTP\*
  - Nucleotides are also available in the Deoxynucleoside Triphosphate Set\*

#### For determination of labeling efficiency

- *i* See section, **Working Solution** for additional information on how to prepare solutions.
- Whatman 3MM paper
- Nylon membranes, positively charged\*
- Anti-Digoxigenin-AP, Fab fragments\*
- CSPD\*
- DIG Wash and Block Buffer Set\* or
  - Washing buffer
  - Maleic acid buffer
  - Detection buffer
  - Blocking solution
- 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\*
- Poly(A)\*
- Poly(dA)
- 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 Tailing Kit can be used for:

- Dot and slot blotting
- Colony and plaque hybridization
- Northern blotting for abundant messages.
- Southern blotting; not recommended for single-copy gene detection in complex genomes.
- In situ hybridization

In addition to common hybridization techniques, DIG-labeled oligonucleotides are especially useful for screening expression libraries for sequence-specific DNA binding proteins, such as transcription factors.

## 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.
- i 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**

#### **Oligonucleotide tailing**

Solution	Composition/Preparation	Storage and Stability	For use in
DIG-dUTP labeling mixture	Mix 9 volumes of 1 mM DIG-11-dUTP (Vial 3) with 1 volume of the appropriate 100 mM deoxynucleotide triphosphate dilution, or 100 mM dNTP mixture.	_	Labeling of oligonucleotide.

#### **Determination of labeling efficiency**

Solution	Composition/Preparation	Storage and Stability	For use in
Washing buffer	0.1 M maleic acid, 0.15 M NaCl, pH 7.5 (+15 to +25°C), 0.3% (v/v) Tween 20*	Store at +15 to +25°C.	Removal of unbound antibody.
Maleic acid buffer	0.1 M maleic acid, 0.15 M NaCl; adjust with NaOH (solid) to pH 7.5 (+15 to +25°C)		Dilution of blocking solution.
Detection buffer	0.1 M Tris-HCI*, 0.1 M NaCl, pH 9.5 (+15 to +25°C)		Adjustment of pH to 9.5.
Blocking stock solution, 10x conc.	Dissolve Blocking Reagent* 10% (w/v) in Maleic acid buffer under constant stirring on a heating block (+65°C), or heat in a microwave oven and autoclave. <i>i</i> The solution remains opaque.	Store at +2 to +8°C initially; after first usage, store in aliquots at $-15$ to $-25$ °C.	Preparation of blocking solution.
Blocking solution, 1x conc.	Dilute the 10x Blocking solution 1:10 in Maleic acid buffer.	Always prepare fresh.	Blocking of nonspecific binding sites on the membrane.
Antibody solution	<ul> <li>Centrifuge the antibody for 5 minutes at 10,000 rpm in the original vial prior to each use, and pipette the necessary amount carefully from the surface.</li> <li>Dilute Anti-Digoxigenin-AP, Fab fragments* 1:10,000 (75 mU/ml) in Blocking solution.</li> </ul>	Store at +2 to +8°C.	Binding to the DIG-labeled probe.

*i* The Washing buffer, Maleic acid buffer, Blocking solution, and Detection buffer are available DNase- and RNase-free in the DIG Wash and Block Buffer Set.

## 2.2. Protocols

#### **Overview**

① Oligonucleotide tailing.

(2) Determination of labeling efficiency.

(3) Hybridization

(4) Post hybridization washes.

#### (5) Immunological detection.

#### Oligonucleotide tailing with DIG-11-dUTP/dATP

In the standard assay described below, using 100 pmol 3'-OH ends (1 µg of a 30-mer oligonucleotide), virtually all of the applied oligonucleotide is tailed as judged from a shift of the unlabeled molecule to the labeled form in polyacrylamide gel electrophoresis (PAGE). Control and standard assay HPLC or gel-purified oligonucleotides can be tailed according to the standard reaction procedure.

## ▲ Do not increase the amount of oligonucleotide in the tailing reaction. To label larger amounts of oligonucleotide, increase the reaction volume and all components proportionally.

Add 100 pmol oligonucleotide to a reaction vial and autoclaved, double-distilled water to a final volume of 9 µl.
 For the control reaction, add 5 µl Control Oligonucleotide, unlabeled (Vial 6) and 4 µl autoclaved, double-distilled water to a reaction vial.

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

Reagent	Volume [µl]
Reaction Buffer (Vial 1)	4
CoCl <sub>2</sub> -solution (Vial 2)	4
DIG-11-dUTP solution (Vial 3)	1
dATP Solution (Vial 4)	1
400 U Terminal Transferase (Vial 5)	1

- Mix and centrifuge briefly.

- Incubate at +37°C for 15 minutes, then place on ice.

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.

#### Tailing with nucleotides other than dATP

Oligonucleotides can also be tailed with DIG-11-dUTP and 100 mM dGTP, or dCTP, or dTTP, or a mixture of all four unlabeled dNTPs.

#### **Tail length**

The tail length reflects the number of incorporated DIG molecules and therefore, the labeling efficiency and sensitivity of the reaction products. Tail length is dependent on the type and concentration of deoxynucleoside triphosphate and the ratio of DIG-11-dUTP to unlabeled nucleotides.

The tail length decreases in the order dATP>dTTP>dGTP>dCTP>dNTP.

Tail length and corresponding DIG-11-dUTP incorporation in the standard assay is shown in the following table. Data for digoxigenin content and average values.

DIG-11-dUTP/NTP labeling mixture, 1:10	dATP	dGTP	dCTP	dTTP	dNTP
Average tail length	50	15	25	10	5
Range of tail length	10 – 100	10 – 25	10 – 40	1 – 20	1 – 10
DIG-11-dUTP/tail	5	1.5	2.5	1	0.5

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 6) and 5 µl sterile, double-distilled water to a reaction vial.

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

Reagent	Volume [µl]
Reaction Buffer (Vial 1)	4
CoCl <sub>2</sub> -solution (Vial 2)	4
DIG-11-dUTP/dNTP tailing mixture	1
400 U Terminal Transferase (Vial 5)	1

- Mix and centrifuge briefly.

- Incubate at +37°C for 15 minutes, then place on ice.

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

- Proceed to section, **Determination of labeling efficiency**.

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

#### Generation of short tail

When less sensitive probes are sufficient for the intended use of the probes, the flexibility of this kit also allows the addition of a very short tail of 2 to 3 nucleotides consisting of DIG-11-dUTP only.

Add 100 pmol oligonucleotide to a reaction vial and autoclaved, double-distilled water to a final volume of 10 µl.
 For the control reaction, add 5 µl Control Oligonucleotide, unlabeled (Vial 6) and 5 µl autoclaved, double-distilled water to a reaction vial.

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

Reagent	Volume [µl]
Reaction Buffer (Vial 1)	4
CoCl <sub>2</sub> -solution (Vial 2)	4
DIG-11-dUTP solution (Vial 3)	1
400 U Terminal Transferase (Vial 5)	1

- Mix and centrifuge briefly.

- Incubate at +37°C for 15 minutes, then place on ice.

Stop the reaction by adding 2 μl 0.2 M EDTA (pH 8.0).
 Proceed to section, Determination of labeling efficiency.

 $\it v$  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 most important for optimal and reproducible hybridization results. Quantification of labeled probes is performed via a direct detection method (spot test), where a series of dilutions of the tailed oligonucleotides is compared to a tailed oligonucleotide standard (Vial 7):

On a nylon membrane, positively charged\*, spot a series of defined dilutions of the Oligonucleotide, DIG-11-dUTP tailed (Vial 7), used as a standard.

- Spot a series of defined dilutions of the tailed olignucleotide, using one row of dilutions for each tailed oligonucleotide.

(2) The nylon membrane is subjected to immunological detection with Anti-digoxigenin-AP conjugate and the premixed stock solution of CSPD, ready-to-use\*.

- The intensities of the dilution series of DIG-labeled oligonucleotide and Control Oligonucleotide (Vial 7) are compared by exposure to an appropriate imager or X-ray film or Lumi-Film.

#### **Dilution series**

Compare a series of dilutions of your labeled oligonucleotide to a series of dilutions of the labeled oligonucleotide (Vial 7). Dilute your labeled oligonucleotide (assumed 100 pmol/22  $\mu$ l) to a starting concentration of 2.5 pmol/ $\mu$ l (original concentration of Vial 7).

Tube No.	Oligo [µl]	From tube No.	DNA Dilution buffer (Vial 10) [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

Prepare the dilutions as described in the following table:

#### **Direct detection**

*i* The volumes suggested below are for a 100 cm<sup>2</sup> membrane processed in a small plastic container, such as a petri dish.

🛕 Use sufficient buffer volumes to cover the membrane completely during all steps.

Apply a 1 µl spot of Tubes 2 to 6 from your labeled oligonucleotide and the labeled control to the nylon membrane.

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

Transfer the membrane into a plastic container with 20 ml Washing buffer.
 Incubate under shaking for 2 minutes at +15 to +25°C.

4 Incubate for 30 minutes in 10 ml Blocking solution.

5 Incubate for 30 minutes in 10 ml Antibody solution.

6 Wash 2 × 15 minutes with 10 ml Washing buffer.

7 Equilibrate 2 to 5 minutes in 10 ml Detection buffer.

B Place membrane with DNA side facing up on a development folder or Hybridization Bag\* and apply 0.1 ml CSPD ready-to-use (4 drops) to the membrane.

**A** Immediately cover the membrane with the second sheet of the folder to spread the substrate evenly and without air bubbles over the membrane.

- Incubate for 5 minutes at +15 to +25°C.

9 Squeeze out excess liquid and seal the edges of the development folder.

#### A Drying of the membrane during exposure will result in dark background.

D Expose using an imaging instrument for 5 to 20 minutes, or to X-ray film or Lumi-Film.

*Luminescence continues for at least 48 hours. The signal increases in the first few hours after initiation of the detection reaction until it reaches a plateau where signal intensity remains almost constant during the next 24 to 48 hours. Multiple exposures can be taken to achieve the desired signal strength.* 

#### Hybridization of tailed probes

Hybridization of oligonucleotides requires hybridization conditions adapted to the length and sequence of the oligonucleotide. There are a number of rules and equations in the literature for the estimation of the melting temperature (Tm) of the oligonucleotide. Application of tailed probes in hybridization experiments can sometimes cause nonspecific hybridization of the tail to related homologous sequences in the target DNA. Therefore, if DIG-11-dUTP/dATP tailed probes are applied, block these sequences by an excess of unlabeled Poly(A)\* and, optionally, Poly(dA) in the hybridization solution.

#### A Do not use formamide in hybridization experiments with oligonucleotides.

#### Hybridization temperature

For oligonucleotides <18 nt, multiply the number of G and C by +4°C and the number of A and T by +2°C.

2 Add the two results; the sum gives the Tm-value.

A more elaborate equation takes into account the ionic strength, G/C content, and length of the oligonucleotide. It is suitable for oligonucleotides from 14 to 70 nucleotides:

 $Tm = 81.5 + 16.6 (log_{10} [Na^+]) + 0.41 (\% G+C) - (600/N)$ 

- N is the length of the oligonucleotide and [Na<sup>+</sup>] is the concentration of sodium ions in the final stringent wash solution.

- Hybridization is carried out at temperatures +5 to +10°C below the Tm-value. However, since the equations mentioned above provide only a guideline for the optimal hybridization temperature, the hybridization conditions must be determined empirically, taking the calculated hybridization temperature as a starting point.

A Tailed oligonucleotides can alter the Tm significantly.

The following steps describe the hybridization procedure with DIG-labeled oligonucleotide probes.

#### A Do not use open trays when working with DIG Easy Hyb buffer.

A For tailed oligonucleotides, add 0.1 mg/ml Poly(A) and 5 μg/ml Poly(dA) to the prehybridization and hybridization to prevent nonspecific hybridization signals caused by the tails.

Pre-heat appropriate volume of DIG Easy Hyb (approximately 20 ml/100 cm<sup>2</sup>) to hybridization temperature.

2 Incubate the membrane for at least 30 minutes with gentle agitation.

႔ Immerse the membrane completely in the DIG Easy Hyb buffer.

3 Add 0.1 to 10 pmol tailed oligonucleotide/ml to pre-heated DIG Easy Hyb ( $\geq$  3.5 ml/100 cm<sup>2</sup> membrane).

4 Pour off prehybridization solution and immediately add probe/DIG Easy Hyb mixture to membrane.

🛕 Avoid foaming as bubbles may lead to background.

**5** Incubate with gentle agitation for 2 hours to overnight at hybridization temperature.

#### 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.

#### 1 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, they should not be allowed to dry out; store membranes immersed in 2x SSC or Maleic acid buffer.

#### Immunological detection

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**

Oligonucleotides tailed according to the described protocol allow the detection of 1 pg homologous DNA in a dot or Southern blot.

## 3. Additional Information on this Product

## 3.1. Test Principle

#### Labeling principle

Oligonucleotides are enzymatically labeled at their 3' end with terminal transferase either by incorporation of a single digoxigenin-labeled dideoxyuridine-triphosphate (DIG-11-ddUTP\*) or by the addition of a longer nucleotide tail. For the generation of tailed oligonucleotide probes, a mixture of deoxynucleotidetriphosphates (dNTPs) and DIG-11-dUTP is used in a template-independent reaction (Fig. 1).





## 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			
<i>i</i> 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.		
<b>1 2 3</b> 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

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.

## 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		
Tris hydrochloride	500 g	10 812 846 001
Buffers in a Box, Premixed SSC Buffer, 20x	4 L	11 666 681 001
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm <sup>2</sup> )	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
CSPD, ready-to-use	2 x 50 mL	11 755 633 001
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
Poly(A)	100 mg	10 108 626 001
dATP	250 μL, 25 μmol, 100 mM 6,250 standard PCR assays of 20 μl each.	11 934 511 001
	1,250 μL, 125 μmol, 100 mM 31,250 standard PCR assays of 20 μl each.	11 969 013 001
dCTP	250 μL, 25 μmol, 100 mM 6,250 standard PCR assays of 20 μl each.	11 934 520 001
dGTP	250 μL, 25 μmol, 100 mM 6,250 standard PCR assays of 20 μl each.	11 934 538 001
	1,250 μL, 125 μmol, 100 mM 31,250 standard PCR assays of 20 μl each.	11 969 030 001
dTTP	250 μL, 25 μmol, 100 mM 6,250 standard PCR assays of 20 μl each.	11 934 546 001
Deoxynucleoside Triphosphate Set	4 x 250 μL, 4 x 25 μmol, 100 mM	11 969 064 001
	4 x 1,250 μL, 4 x 125 μmol, 100 mM	03 622 614 001

## 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.



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