For general laboratory use.

dUTP
PCR Grade, sodium salt

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Cat. No. 11 934 554 001	250 µl 25 µmol, 100 mM 6,250 standard PCR assays of 20 µl each.
Cat. No. 11 969 056 001	1,250 µl 125 µmol, 100 mM 31,250 standard PCR assays of 20 µl each.
Cat. No. 03 732 720 001	4 x 1,250 µl 4 x 125 µmol, 100 mM 125,000 using standard PCR assays of 20 µl each.

Store the product at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	dUTP, PCR Grade, Na-salt	▪ Deoxynucleotide of high purity, specially manufactured and tested for PCR and RT-PCR. ▪ Clear, colorless, 100 mM dUTP salt solution in water, pH 8.3.	11 934 554 001	1 vial, 25 µmol (250 µl)
			11 969 056 001	1 vial, 125 µmol (1,250 µl)
			03 732 720 001	4 vials, 125 µmol (1,250 µl) each

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	dUTP, PCR Grade	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Autoclaved reaction tubes for preparing PCR mixes and dilutions
- PCR reaction vessels, such as 0.2 ml thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler

For PCR

- Primers
- Template DNA
- Water, PCR Grade*
- Uracil DNA-Glycosylase*
- Taq DNA Polymerase*
- dNTPs, PCR Grade or
- PCR Grade Nucleotide Mix*
- PCR buffer, 10x conc. with $MgCl_2$
- Mineral oil (optional)

1.4. Application

Use dUTP, PCR Grade in:

- PCR
- RT-PCR
- Carryover prevention, see section, **Protocols**.

2. How to Use this Product

2.1. Before you Begin

Mg^{2+} Concentration

2.5 mM

General Considerations

The optimal conditions, including times and temperatures, concentration of enzyme, template DNA, and Mg^{2+} vary from system to system and must be determined for each experimental system. At the very least, titrate the Mg^{2+} concentration and the amount of enzyme used per assay to ensure optimal efficiency of DNA synthesis.
As a starting point, use the following guidelines:

- Optimal Mg^{2+} concentration can vary from 1.5 mM to 5 mM. In most cases, a Mg^{2+} concentration of 2.5 mM will produce satisfactory results.
- dNTP concentration: always use equal concentrations of all four dNTPs. The final concentration of each dNTP should be between 50 and 500 µM. The most commonly used concentration is 200 µM. Increase concentrations of Mg^{2+} when increasing the concentration of dNTP.
- The replacement of dTTP by dUTP allows the application of the carryover prevention technique to reduce the number of false positive results in PCR and RT-PCR. The dUTP concentration should be increased to 0.6 mM for optimal PCR efficiency. Increased dUTP concentration requires an increased $MgCl_2$ concentration. In many applications a concentration of 2.5 mM $MgCl_2$ should be sufficient; further optimization might be necessary.

Working Solution

Deoxynucleotide mix (dNTP)

Prepare a mix containing 10 mM each dATP, dCTP, dGTP and 30 mM dUTP.
For example, for the preparation of 100 µl dNTP mix, add 10 µl each dATP, dCTP, dGTP and 30 µl dUTP to 40 µl Water, PCR Grade*.

2.2. Protocols

Carryover prevention using dUTP and Uracil DNA-Glycosylase (UNG)

Preparation of master mix

See section, **Working Solution** *for additional information on preparing solutions.*
For a larger number of reactions, prepare a master mix which contains buffer, primers, and Taq DNA Polymerase.

1. Thaw components listed below and place them on ice.

2. Briefly vortex and centrifuge all reagents before setting up the reactions.

3 To a autoclaved, nuclease-free reaction tube on ice, add the components in the order listed for each 100 µl reaction:

Reagent	Volume [µl]	Final conc.
dNTP mix	2	200 µM of each dNTP 600 µM dUTP
Primer mixture	0.5 – 5	0.1 to 1.0 µM each
Taq DNA polymerase*, 5 U/µl	0.5	2.5 U
PCR buffer, 10x conc. with $MgCl_2$	10	1x (2.5 mM $MgCl_2$)
Uracil DNA-Glycosylase, heat-labile*	2	2 U
Template DNA	10	variable
Water, PCR Grade*	to make a final volume of 100 µl	–
Final Volume	**100**	

4 Mix and centrifuge briefly to collect the solution at the bottom of the tube.

PCR

i The following thermal profile is an example. Different thermal cyclers may require different profiles.

1 Overlay the reaction carefully with mineral oil if required by the thermal cycler.

2 Place the samples in a thermal block cycler and perform PCR.

Step	Temperature [°C]	Time	Number of Cycles
UNG incubation	20	1 – 10 min	1
UNG inactivation/cleavage at abasic sites	95	2 min	1
UNG denaturation	95	2 min	1
Denaturation Annealing(1) Elongation(2)	95 60 72	45 sec 60 sec 2 min	25 – 35

3 Analyze the PCR products by agarose gel electrophoresis.

(1) Optimal annealing temperature depends on individual primers and template used and must be optimized.
(2) The elongation step in the last cycle can be prolonged to 10 minutes to ensure completion of the reaction.

2.3. Parameters

Purity

High purity deoxynucleotide, specially manufactured and tested for PCR and RT-PCR.
dUTP (HPLC), area >99%
dUDP (HPLC), area <0.9%

3. Additional Information on this Product

3.1. 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

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
Taq DNA Polymerase, 5 U/µl	100 U, 5 U/µl 80 reactions	11 146 165 001
	500 U, 5 U/µl 400 reactions	11 146 173 001
	4 x 250 U, 5 U/µl 800 reactions	11 418 432 001
	10 x 250 U, 5 U/µl 2,000 reactions	11 596 594 001
	20 x 250 U, 5 U/µl 4,000 reactions	11 435 094 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001

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 and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For general laboratory use.

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

1221.11977326001 ®

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