

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



# Biotin-16-dUTP

 **Version: 10**

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Biotin- $\epsilon$ -aminocaproyl- $\gamma$ -aminobutyryl-[5-(3-aminoallyl)]-2'-deoxy-uridine-5'-triphosphate]  
tetralithium salt

**Cat. No. 11 093 070 910**    50 nmol  
50  $\mu$ l, 1 mM

**Store the product at  $-15$  to  $-25^{\circ}\text{C}$ .**

|           |                                                                |          |
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# 1. General Information

## 1.1. Contents

| Vial / Bottle | Label          | Function / Description           | Content          |
|---------------|----------------|----------------------------------|------------------|
| 1             | Biotin-16-dUTP | 1 mM tetralithium salt solution. | 1 vial,<br>50 µl |

## 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             | Biotin-16-dUTP | Store at –15 to –25°C.<br> <b>A decomposition of approximately 5% may occur within 6 months.</b> |

## 1.3. Additional Equipment and Reagent required

### For random primed DNA labeling reaction

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

- Hexanucleotide Mix\*, 10x conc.
- Biotin/dNTP\* mixture, 10x conc.

 Also available as a *Set of Deoxynucleotides, PCR Grade\**.

- Klenow Enzyme\*, 100 U
- EDTA, 0.2 M, pH 8.0
- Autoclaved, double-distilled water
- Water bath
- Ice bath

### Analysis of biotin-labeled DNA

- Nylon Membranes\*
- Streptavidin-AP-conjugate for nucleic acid detection\* in combination with:
- Chemiluminescent substrate (CSPD\*, CDP-Star\*), or a
- Color substrate (NBT/BCIP\*)

## 1.4. Application

Biotin-16-dUTP can be used for the following applications:

- Nonradioactive DNA labeling, such as random priming, PCR labeling, tailing, or nick translation. Biotin-16-dUTP replaces dTTP in the random primed DNA labeling reaction or in nick translation in a ratio of 35% Biotin-16-dUTP and 65% dTTP, as well as in the PCR.
- Substrate for DNA Polymerase I (holoenzyme and Klenow fragment)\*, Taq DNA Polymerase\*, Terminal Transferase (3'-end labeling), and Reverse Transcriptase (Transcriptor Reverse Transcriptase)\*.

## 2. How to Use this Product

### 2.1. Before you Begin

#### Sample Materials

Biotin-16-dUTP is used with linearized DNA.

#### Working Solution

#### Working solutions for random primed DNA labeling reaction

| Reagent/Buffer                  | Composition/Concentration |
|---------------------------------|---------------------------|
| Biotin/dNTP* mixture, 10x conc. | 1 mM dATP                 |
|                                 | 1 mM dGTP                 |
|                                 | 1 mM dCTP                 |
|                                 | 0.65 mM dTTP              |
|                                 | 0.35 mM Biotin-16-dUTP    |
|                                 | pH 7.5 (+20°C)            |

### 2.2. Protocols

#### Random primed DNA labeling reaction

The following protocol describes a labeling reaction using Biotin-16-dUTP.

**i** Larger amounts can be labeled by scaling up of all components and volumes. Linear DNA is labeled more efficiently than circular and supercoiled DNA.

**1** Purify the linearized DNA to be labeled using the High Pure PCR Product Purification Kit\* or phenol chloroform extraction and ethanol precipitation.

**2** To a reaction vial, add 10 ng to 3 µg DNA and autoclaved, double-distilled water to a final volume of 15 µl.

**3** Denature the DNA by heating in a boiling water bath for 10 minutes at +95°C; quickly chill in an ice/water bath.

**i** Full denaturation is essential for efficient labeling.

**4** Add the following to the freshly denatured probe on ice:

| Reagent                        | Volume [µl] |
|--------------------------------|-------------|
| Hexanucleotide Mix, 10x conc.  | 2           |
| Biotin/dNTP mixture, 10x conc. | 2           |
| Klenow Enzyme                  | 1           |

– Mix and centrifuge briefly.

– Incubate for at least 60 minutes at +37°C.

**⚠ Longer incubations up to 20 hours increase the yield of labeled DNA.**

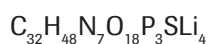
**5** Stop the reaction by adding 2 µl 0.2 M EDTA (pH 8.0).

## Analysis of biotin-labeled DNA

Analyze the biotin-labeled DNA prior to usage as a hybridization probe via spot assay in combination with Nylon Membranes\* and detection with the Streptavidin-AP-conjugate for nucleic acid detection\*.

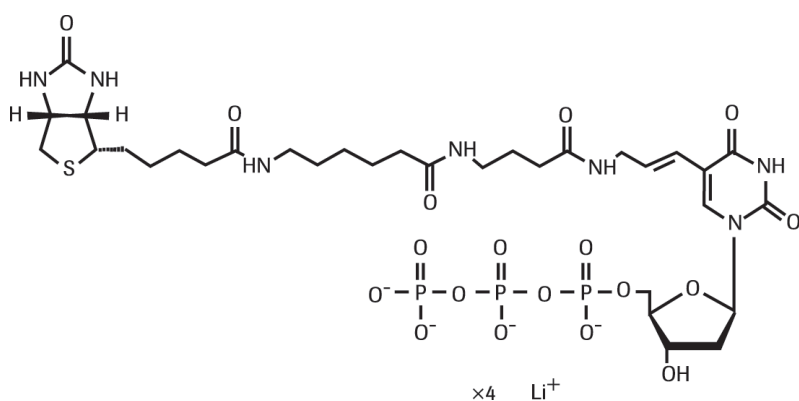
## 2.3. Parameters

### Chemical Formula



### Chemical Name

### Structural formula



**Fig. 1:** Chemical structure of Biotin-16-dUTP

### Molecular Weight

971.5 Da

## 3. Supplementary Information

### 3.1. 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>Information Note: Additional information about the current topic or procedure.</i>                      |
|  | <b>Important Note: Information critical to the success of the current procedure or use of the product.</b> |
| 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.                                           |

### 3.2. Changes to previous version

Layout changes.

Editorial changes.

### 3.3. Ordering Information

| Product                                              | Pack Size                                                    | Cat. No.       |
|------------------------------------------------------|--------------------------------------------------------------|----------------|
| Reagents, kits                                       |                                                              |                |
| 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 |
| 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 |
| Streptavidin-AP-conjugate for nucleic acid detection | 150 U, (200 µl)                                              | 11 093 266 910 |
| Taq DNA Polymerase, 5 U/µl                           | custom fill, 5 U/µl                                          | 11 147 633 103 |
| Transcriptor Reverse Transcriptase                   | custom fill                                                  | 03 531 252 103 |
| Terminal Transferase, recombinant                    | custom fill                                                  | 03 289 869 103 |
| Taq DNA Polymerase, 1 U/µl                           | 250 U, 1 U/µl, 200 reactions in a final volume of 50 µl      | 11 647 679 001 |
|                                                      | 1,000 U, 4 x 250 U, 800 reactions in a final volume of 50 µl | 11 647 687 001 |
| Hexanucleotide Mix                                   | 100 µl, 10x conc., 50 labeling reactions                     | 11 277 081 001 |
| dATP, PCR Grade                                      | 20 ml, 2,000 µmol                                            | 04 631 056 103 |
|                                                      | 100 ml, 10,000 µmol                                          | 11 889 516 103 |
| dCTP, PCR Grade                                      | 20 ml, 2,000 µmol                                            | 04 631 072 103 |
|                                                      | 100 ml, 10,000 µmol                                          | 11 889 508 103 |
| dGTP, PCR Grade                                      | 20 ml, 2,000 µmol                                            | 04 631 129 103 |
|                                                      | 100 ml, 10,000 µmol                                          | 11 889 524 103 |
| dTTP, PCR Grade                                      | 20 ml, 2,000 µmol                                            | 04 631 137 103 |
|                                                      | 100 ml, 10,000 µmol                                          | 11 889 559 103 |
| 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 |

### 3.4. Trademarks

All product names and trademarks are the property of their respective owners.

### 3.5. License Disclaimer

For patent license limitations for individual products please refer to:

**List of biochemical reagent products.**

### 3.6. Regulatory Disclaimer

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

### 3.7. Safety Data Sheet

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

### 3.8. 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.

