FT-595911


# Biotin-X-dUTP

*Biotin-X-dUTP can be enzymatically incorporated into DNA via nick translation, PCR random priming or 3'-end terminal labeling.*

## Description

| Product name<br>cat.number | MW<br>(g·mol$^{-1}$) | Comments |
|---|---|---|
| **Biotin-11-dUTP, 1mM in pH 7.5 Tris-HCl buffer, 1 mM EDTA**<br>FP-AM539B, 100 µl | 886.5 | - Biotin-11-2'-deoxyuridine-5'-triphosphate, tetralithium salt<br>- The length of linker "11" is optimal for the majority of applications. |
| **Biotin-11-dUTP, lyophilized powder**<br>FP-AY489A, 50 µg | 886.5 | - Biotin-11-2'-deoxyuridine-5'-triphosphate, tetralithium salt<br>- Lyophilized solid that is more suitable for long term storage. The product contains lyophilized TE buffer, so you only need to add an appropriate amount of deionized $H_2O$ to reconstitute the solution. |
| **Biotin-16-dUTP, 1mM in pH 7.5 Tris-HCl buffer**<br>FP-AM541A, 50 µl | 971.5 | - Biotin-16-2'-deoxyuridine-5'-triphosphate, tetralithium salt<br>- The terminal deoxynucleotidyl transferase (TDT)-mediated biotin-dUTP nick end-labeling (TUNEL) method has been commonly used for apoptosis studies. |
| **Biotin-16-dUTP, lyophilized powder**<br>FP- AM542A, 50 µg | 971.5 | - Biotin-16-2'-deoxyuridine-5'-triphosphate, tetralithium salt<br>- Lyophilized solid that is more suitable for long term storage. The product contains lyophilized TE buffer, so you only need to add an appropriate amount of deionized $H_2O$ to reconstitute the solution. |
| **Biotin-20-dUTP, 1mM in pH 7.5 Tris-HCl buffer**<br>FP-AM543B , 50 µl | 1020.54 | - Biotin-20-2'-deoxyuridine-5'-triphosphate, tetralithium salt<br>- Longer and water-soluble spacer group, which should facilitate interaction between the biotin group and avidin or streptavidin. |
| **Biotin-20-dUTP, lyophilized powder**<br>FP-AM543A , 50 µg | 1020.54 | - Biotin-20-2'-deoxyuridine-5'-triphosphate, tetralithium salt<br>- Lyophilized solid that is more suitable for long term storage. The product contains lyophilized TE buffer, so you only need to add an appropriate amount of deionized $H_2O$ to reconstitute the solution. |

**Storage:** –20°C >1 year Protect from light

## Introduction

The number 'X' is the number of atoms in the linker between biotin and dUTP. The length of the linker affects the incorporation efficiency of the biotin-dUTP probe into DNA using DNA polymerases, and it also affects biotin/avidin or biotin/streptavidin. In general, the shorter the linker, the more efficiently the biotin-dUTP is


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incorporated into DNA by DNA polymerases. On the other hand, the longer the linker, the better biotin can interact with avidin or streptavidin.

## Standard protocol

The most popular approach for DNA PCR-labeling with labeled dUTP is based on the usage of dNTPs mixture which contains labeled dUTP and all 4 other dNTP in regular concentrations. The molar ratio dUTP/labeled dUTP (or dTTP/labeled dUTP) can vary from 3 :1 to 1 :1. The incorporation efficiency depends mainly on the usage of dTTP or dUTP (the incorporation efficiency of dTTP is slightly better than those for dUTP) and on the enzyme used for PCR. Regular Taq DNA polymerase incorporates dUTP (and especially labeled dUTP) less efficient than Taq DNA polymerase with modified active center.
In some special applications one may completely substitute dTTP by labeled dUTP and to get DNA with all « T » substituted to labeled dUTP. Meanwhile, this 100% labeled DNA will be quite different from regular DNA in terms of electrophoresis mobility, hydrophobic properties, denaturation behavior etc. If all these points can be neglected , one can completely substitute dTTP by labeled dUTP.

The regular protocol for DNA labeling by PCR use a ratio of 1:2 (labeled dUTP : non-labeled dTTP) :

| Reagent | Final concentration | Quantity for 50 µl of reaction mixture |
|---|---|---|
| Sterile deionized water | - | Variable |
| 10X PCR buffer | 1X | 5 µl |
| 10 mM dNTP Mix | 0.2 mM of each | 1 µl |
| labeled dUTP, 1 mM | 0.1 mM | 5 µl |
| Primer I | 0.1 – 1 µM | Variable |
| Primer II | 0.1 – 1 µM | Variable |
| Taq DNA Polymerase | 1.25 U – 2.5 U/50 µl | 1.25 U – 2.5 U |
| 100 mM $MgCl_2$ | 1 – 4 mM | Variable |
| Template DNA | 10 pg – 1 µg | Variable |

PCR should be performed as optimized on the regular dNTPs – with the same $MgCl_2$ concentration, with the temperatures and cycles optimized for the particular template and primers.

Protocol with Biotin-dUTP, PCR grade

| 20 µl PCR labeling assay | | | |
|---|---|---|---|
| **Component** | **Stock conc.** | **Amount** | **Final conc.** |
| High yield buffer without $MgCl_2$ (Cat.-No. PCR-201) | 10x | 2 µl | 1x |
| $MgCl_2$ stock solution | 25 mM | 1.6 µl | 2 mM |
| dATP | 1 mM | 2 µl | 100 µM |
| dCTP | 1 mM | 2 µl | 100 µM |
| dGTP | 1 mM | 2 µl | 100 µM |
| dTTP | 1 mM | 1 µl | 50 µM |
| Biotin-dUTP-PCR | 1 mM | 1 µl [1)] | 50 µM [1)] |
| forward Primer | 10 µM | 1 µl | 500 nM |
| reverse Primer | 10 µM | 1 µl | 500 nM |
| Template DNA | | 0.1-10 ng | 5-500 pg/µl |
| Taq Pol (Cat.-No. PCR-201) | 5 units/µl | 0.2 µl (1 unit) | 0.05 units/µl |
| PCR grade $H_2O$ | | Fill up to 20 µl | |


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Recommended cycling conditions

| | | | |
|---|---|---|---|
| Initial denaturation | 94 °C | 2 min | 1x |
| Denaturation | 94 °C | 30 sec | 25-30x |
| Annealing [1)] | 50-60 °C | 30 sec | |
| Elongation [2)] | 72 °C | 1 min | |
| Final elongation | 72 °C | 5 min | 1x |

1) *The annealing temperature depends on the melting temperature of the primers used.*
2) *The elongation time depends on the length of the fragments to be amplified. A time of 2 min/kbp is recommended.*

For optimal amplification results and high incorporation rates an individual optimization of the recommended PCR assay and cycling conditions may be necessary for each new primer-template pair.

## Related products

| Description | Cat. No. |
|---|---|
| UptiTherm DNA Polymerase (1000 Units)<br>*5U/µl with Mg free Buffer + 50 mM MgCl2 buffer* | UPS53921 |
| Terminal deoxynucleotidyl transferase (1000 Units) | HP9020 |
| 2x Hot Start PCR Master Mix (500 Units) | CJ5361 |
| Fast EvaGreen qPCR Master Mix (500 tests) | DV7221 |
| PCR set 1 (dATP, dGTP, dCTP, dTTP 100mM each) | UP968640 |
| PCR Mix 3 (200µl)<br>(10 mM of each dA, dC, dG and dT) | UP984440 |
| GelRed nucleic acid gel prestaining kit | FJ5570 |

## Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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