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

# Biotin-High Prime

**Version: 09**
Content Version: November 2020

For the nonradioactive labeling of DNA with Biotin-16-dUTP, using random oligonucleotides as primers. Premixed solution for 25 labeling assays.

**Cat. No. 11 585 649 910** 100 µl
25 labeling assays

**Store product at −15 to −25°C.**

# 1. General Information

## 1.1. Contents

| Vial / Bottle | Label | Function / Description | Content |
|---|---|---|---|
| 1 | Biotin-High Prime, 5x conc. | Random prime labeling mixture: Premixed solution of 1 U/µl Klenow polymerase, labeling grade, 1 mM dATP, 1 mM dCTP, 1 mM dGTP each, 0.65 mM dTTP, 0.35 mM Biotin-16-dUTP, and 5x stabilized reaction buffer in 50% (v/v) glycerol. | 1 vial, 100 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-High Prime, 5x conc. | Store at −15 to −25°C.<br>⚠ ***Avoid repeated freezing and thawing.***<br>⚠ ***To avoid contamination, aliquot and store the solution in 2 to 3 vials.*** |

## 1.3. Additional Equipment and Reagent required

**For random primed DNA labeling**

- 0.2 M EDTA, pH 8.0
- Autoclaved, double-distilled water
- Water bath
- Ice bath

**For labeling DNA isolated from agarose**

- 0.2 M EDTA, pH 8.0
- Autoclaved, double-distilled water
- Water bath

**For detection of biotin-labeled DNA**

- CSPD*
- CDP-*Star**
- NBT/BCIP*

## 1.4. Application

Biotin-High Prime-labeled probes are used in a variety of hybridization reactions:

- Southern blots
- Northern blots
- Dot/slot blots
- Screening of gene libraries
- *In situ* hybridizations

# 2. How to Use this Product

## 2.1. Before you Begin

### Sample Materials

#### Templates for labeling reaction

- DNA fragments of at least 100 bp.
- Linearized plasmid, cosmid, or λDNA.
- Supercoiled DNA
- Minimal amounts of DNA (10 ng), such as DNA restriction fragments isolated from gels or in molten agarose.

*For the Random primed DNA labeling protocol, 0.01 to 3 µg of template can be labeled.*

### Safety Information

#### For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

### Working Solution

| Solution | Composition | Use | Storage and Stability |
|---|---|---|---|
| Water | Autoclaved, double-distilled water | Dilution of DNA. | Store at +15 to +25°C. |
| EDTA | 0.2 M ethylenediaminetetraacetic acid, pH 8.0 | Stops the reaction. | Store at +15 to +25°C. |

## 2.2. Protocols

#### Random primed DNA labeling

Perform the standard random primed DNA labeling according to the following steps.

1. To a reaction vial, add 1 µg template DNA (linear or supercoiled) and autoclaved, double-distilled water to a final volume of 16 µl.

2. Denature the DNA by heating in a boiling water bath for 10 minutes; quickly chill in an ice/water bath.
   *Full denaturation is essential for efficient labeling.*

3. Mix Biotin-High Prime thoroughly and add 4 µl to the denatured DNA; mix, and centrifuge briefly.
   – Incubate for 1 hour or overnight at +37°C.
   *Longer incubations up to 20 hours increase the yield of Biotin-labeled DNA, see* **Table, Labeling reaction yield**.

4. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0, and/or by heating to +65°C for 10 minutes.

*The length of the Biotin-labeled fragments obtained with Biotin-High Prime range from 200 bp to ≥1,000 bp, depending on the length of the original template.*

## Labeling DNA isolated from low-melting point agarose

1. Excise the DNA fragment to be labeled cleanly from a low-melting point agarose gel and transfer it to a 1.5 ml microfuge tube.

2. Add sterile, double-distilled water to a ratio of 3 ml/g gel and heat the tube for 7 minutes at +100°C to melt the gel and denature the DNA.
   – After cooling to +37°C, the DNA/agarose mixture can be used directly for labeling.

3. Mix Biotin-High Prime thoroughly; add 4 µl, mix, and centrifuge briefly.

4. Incubate overnight at +37°C.

5. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0 and/or by heating to +65°C for 10 minutes.

## Labeling reaction yield

The labeling efficiency is shown in the following table and in Figure 1.

*Reactions were performed with increasing amounts of different template DNAs for 1 hour and 20 hours. The yield of biotin-labeled DNA was determined by the incorporation of a radioactive tracer and confirmed by a dot blot. Numbers shown are the average of independent labeling assays.*

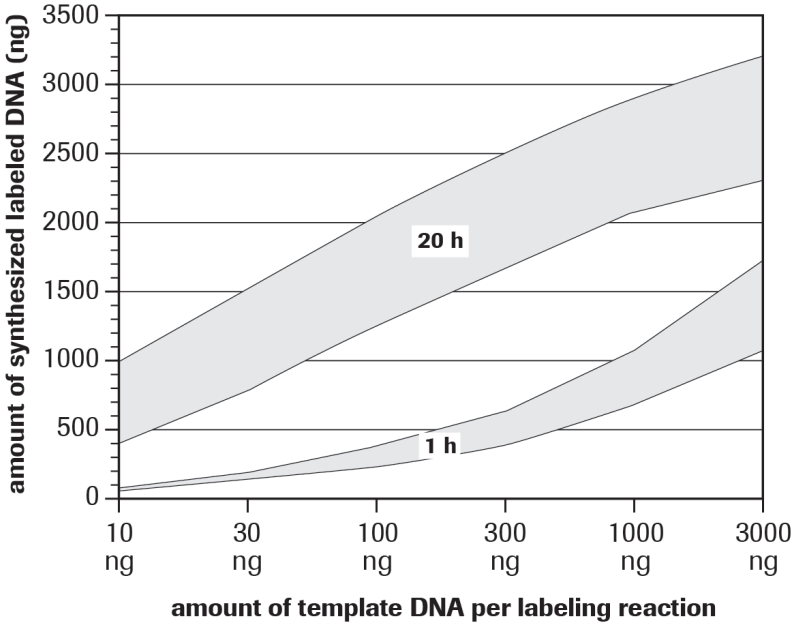


**Fig. 1:** Yield of biotin-labeled DNA from different amounts of template DNAs for 1 hour and 20 hours incubation of the Biotin-High Prime reaction at +37°C.

## Detection of biotin-labeled DNA

Biotin-labeled DNA is detected by streptavidin conjugated to the enzyme alkaline phosphatase which catalyzes a color reaction with 5-bromo-4-chloro-3-indolyl-phoshate and 4-nitro blue tetrazolium chloride (NBT/BCIP*) or by a chemiluminescent reaction with CSPD* or CDP-*Star**. Alternatively, especially for *in situ* applications, biotin-labeled hybrids can also be detected by streptavidin conjugated to different fluorochromes.

## 2.3. Parameters

### Chemical Name

#### Structural formula

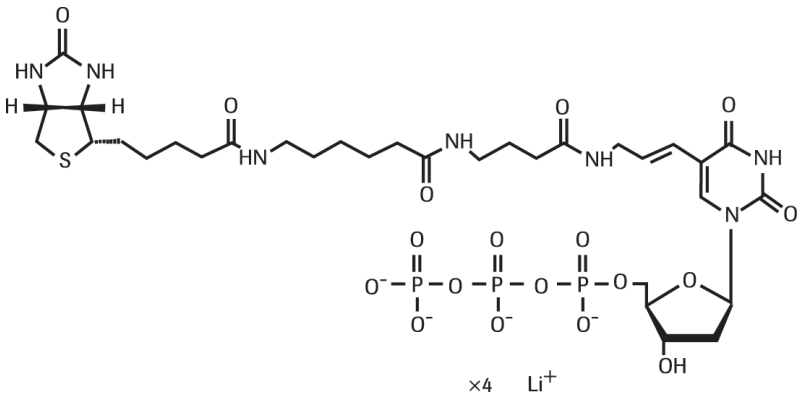


**Fig. 2:** Structure of Biotin-16-dUTP.

# 3. Additional Information on this Product

## 3.1. Test Principle

Biotin-labeled DNA probes are generated with Biotin-High Prime according to the random primed labeling technique.

① The complementary DNA strand is synthesized by Klenow polymerase using the 3'OH termini of the random oligonucleotides as primers.

② Biotin-16-dUTP is incorporated into the newly synthesized complementary DNA strand.
– The premixed Biotin-High Prime is ideal for for convenient and efficient nonradioactive labeling of DNA with biotin.

# 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.
New information added related to the REACH Annex XIV.

## 4.3. 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 |
| NBT/BCIP Stock Solution | 8 ml | 11 681 451 001 |

## 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**.

## 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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