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

# Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester

**Version: 19**
Content Version: June 2021

**Cat. No. 11 333 054 001** 5 mg

**Store product at −15 to −25°C.**

# 1. General Information

## 1.1. Contents

| Vial / Bottle | Label | Function / Description | Content |
|---|---|---|---|
| 1 | Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester | Lyophilized, white powder | 1 vial, 5 mg |

## 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 | Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic-acid-N-hydroxysuccinimide ester | Store at −15 to −25°C. |

### Reconstitution

Proceed as described in the Protocols section, **Labeling of oligonucleotides, Labeling of proteins**.

## 1.3. Additional Equipment and Reagent required

#### For labeling of oligonucleotides

- Double-distilled water
- Dimethylformamide (DMF)
- Sodium borate, 100 mM, pH 8.5
- Sodium acetate, 3 M , pH 8.5
- Ethanol, A.R.

*Use fresh DMF to increase labeling efficiency.*

#### For isolation of labeled oligonucleotide

- HPLC column, RP 18, 5 µm, 25 × 0.4 cm
- Buffer A: Triethylammoniumacetate, 100 mM, pH 7
- Buffer B: Triethylammoniumacetate, 100 mM, acetonitrile (1:1), pH 7

#### For labeling of proteins

- Ethanol, A.R.
- Dimethylsulfoxide (DMSO), A.R.
- Phosphate-buffered saline (PBS), pH 8.5

#### For purification of labeled proteins

- Sephadex G25 column

## 1.4. Application

Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester can be used for labeling of:
- Oligonucleotides
- Proteins

# 2. How to Use this Product

## 2.1. Before you Begin

### Working Solution

#### Preparation of working solution for the labeling of proteins

With stirring, dissolve 0.327 mg Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester in 8.18 µl DMSO or ethanol.

## 2.2. Protocols

### Labeling of oligonucleotides

*Typically, the oligonucleotide is prepared by chemical solid phase synthesis. It should be reacted in a final synthesis cycle with, for example, "Aminolink II", generating a 5'-terminal amino function. After cleavage of the protection group by concentrated ammonia, the oligomer solution is concentrated in a vacuum.*

1. Dissolve the solid residue of the crude oligonucleotide in a mixture consisting of 300 µl double-distilled water and 30 µl 3 M sodium acetate.

2. Transfer the solution to a reaction vial, add 0.9 ml ethanol, mix, and chill 2 to 3 hours at −15 to −25°C.

3. Spin 15 minutes at 20,000 × *g* and decant the supernatant.

4. Wash the pellet with 100 µl ice-cold ethanol.

5. Spin 5 minutes at 20,000 × *g* and decant supernatant.

6. Dissolve ethanol precipitated oligonucleotide (approximately 100 nM) in 50 µl 100 mM sodium borate buffer, pH 8.5.

7. Dissolve 2 µmol (1.3 mg) Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester in 50 µl fresh DMF.
   – Add this mixture to the oligonucleotide solution.
   *A clear solution should be obtained. If necessary, add several drops of buffer or DMF to homogenize.*

8. Incubate overnight at +15 to +25°C.

## Isolation of labeled oligonucleotides

*Since the labeling reaction is not quantitative, separation of the labeled oligonucleotide from the unlabeled compound must be performed. This can be done by reverse phase HPLC.*

1. Concentrate the mixture from the labeling reaction under vacuum.
   *Alternatively, perform an ethanol precipitation.*

2. Dissolve the residue in 1 ml double-distilled water and apply onto an HPLC column, RP 18, 5 µm, 25 × 0.4 cm.

3. Perform the elution as follows:

| Buffer | Description |
|---|---|
| A | Triethylammoniumacetate, 100 mM, pH 7 |
| B | Triethylammoniumacetate, 100 mM, acetonitrile (1:1), pH 7 |

   – Use a gradient in 30 minutes to 80% B.

   *The digoxigenin-labeled oligonucleotide is eluted in the last fraction.*

4. Concentrate the appropriate fraction under vacuum and desalt as usual, for example, gelfiltration on Sephadex or dialysis in a SPECTAPOR 1000).

## Labeling of proteins

The following steps describe the labeling of 1 mg polyclonal antibody.

*The molar reaction mixture of antibody: Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester is 1:70.*

1. Dissolve 1 mg polyclonal antibody in 1 ml PBS, pH 8.5.

2. Add Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester working solution, see section **Working Solution**.

3. Incubate 2 hours at +15 to +25°C.

## Purification of labeled proteins

To remove unbound Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester and the solvent, dialyze the protein solution against PBS or use a Sephadex G25 column.

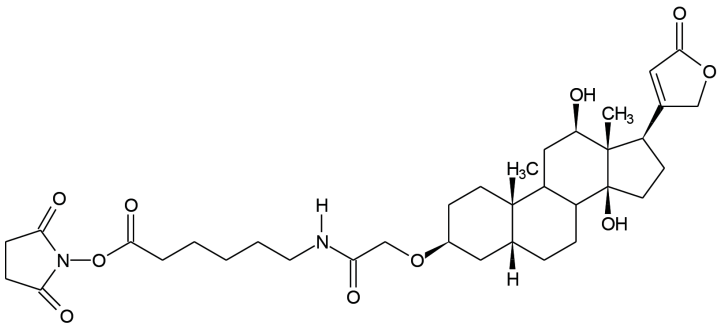
## 2.3. Parameters

### Chemical Formula

$C_{35}H_{50}N_2O_{10}$

### Chemical Name

#### Structural formula



### Molecular Weight

658.8 Da

# 3. Additional Information on this Product

## 3.1. Test Principle

The activated ester reacts with amino groups under mild conditions, therefore the digoxigenin moiety can be introduced, for example, into proteins or 5′-amino substituted oligonucleotides.

# 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.** | |
| ①②③ etc. | Stages in a process that usually occur in the order listed. |
| ❶❷❸ 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. Trademarks

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

## 4.4. License Disclaimer

For patent license limitations for individual products please refer to:
**List of biochemical reagent products**.

## 4.5. Regulatory Disclaimer

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

## 4.6. Safety Data Sheet

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

## 4.7. 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.

0621.11333623001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany