

# D-Biotinoyl- $\epsilon$ -Aminocaproic Acid N-Hydroxysuccinimide Ester

Crystallized

Cat. No. 11 008 960 001 50 mg

Cat. No. 11 008 978 001 250 mg

Version August 2006

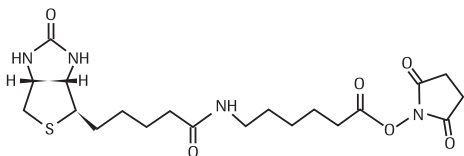
Store at +2 to +8° C

## Product overview

### Commercial availability

White crystals; clear solution in acetonitrile/water 1:1 (c = 50 mg/ml).

### Structural formula



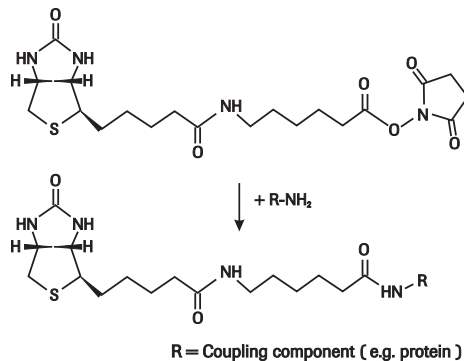
### Properties

Formula	C <sub>20</sub> H <sub>30</sub> N <sub>4</sub> O <sub>6</sub> S
Molecular weight	454.5
Melting range	159–161°C

### Typical analysis

D-Biotinoyl- $\epsilon$ -Aminocaproic Acid N-Hydroxysuccinimide Ester (Biotin-7-NHS) (from elementary analysis)  $\geq 97\%$ .  
TLC: chromatographically homogeneous.

### Labeling principle



### Application

Biotinylation of proteins. The substance can also be used to label 5'-amino-linked oligonucleotides in analogy to the protocol in the pack insert of DIG-NHS ester\* (Cat. No. 11 333 054 001).

### Product description

The activated ester reacts under mild conditions with amino groups. The aminocaproic acid spacer has the advantage of not influencing the binding of strept-avidin/biotin complexes when using biotin-labeled macromolecules.

### Storage and stability

Stable at +2 to +8°C until the expiration date printed on the label.

## Biotinylation of proteins

### Before you begin

- The antibody should be dissolved in 50 mM phosphate buffered saline (PBS), pH 7.5–8.5 (5–20 mg/ml depending on solubility).
- Biotin-7-NHS should be added in a 5–15-fold molar excess. According to our experience start with a 10-fold excess.
- The volume ratio Biotin-7-NHS solution/protein solution should be 1:10 to 1:50.

### Preparation of the Biotin-7-NHS solutions

Biotin-7-NHS should be dissolved in distilled DMF or DMSO (approx. 15–30 mg/ml).  
**Note:** The Biotin-7-NHS solutions should be prepared freshly before use. Use freshly distilled DMF.

### Procedure

Example for biotinylation of proteins using D-Biotinoyl- $\epsilon$ -Aminocaproic Acid N-hydroxysuccinimide Ester.

Step	Action
1	Dissolve 12 mg protein, M <sub>r</sub> 40 kD (= 3 × 10 <sup>-7</sup> mol) in 1 ml PBS, pH 7.5.
2	Add a solution of 1.02 mg Biotin-7-NHS (= 3 × 10 <sup>-6</sup> mol) in 50 $\mu$ l DMF (= 20.4 mg Biotin-7-NHS/ml DMF).
3	Stir at 15 to 25°C for 2–4 hours. <b>Note:</b> During the reaction the pH must not drop under 7.
4	Dialyze against PBS over night at +2 to +8°C; buffer should be changed two or three times.
5	Centrifuge dialysate 8000 × g, 10 min, at 15 to 25°C.
6	Determine protein concentration.
7	Store conjugate at –15 to –25°C after shock-freezing.

### Reference

- 1 Bonhard, C. et al. (1984) *Immunolabelling for Electron Microscopy* (Polak, J. M.; Varndell, J. M., eds.) Elsevier Scientific Publishers Amsterdam, p. 95.

\* available from Roche Applied Science

## Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

**www.roche-applied-science.com/support**

To call, write, fax, or email us, visit the Roche Applied Science home page, [www.roche-applied-science.com](http://www.roche-applied-science.com), and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.



Roche Diagnostics GmbH  
Roche Applied Science  
68298 Mannheim  
Germany