Biotin Protein Labeling Kit

For labeling of proteins with biotin

Cat. No. 11 418 165 001
For 5 labeling reactions

Stability
The kit is stable at +2 to +8°C.

Principle of labeling
Free amino groups of the protein to be labeled react with D-biotinoyl-\(\epsilon\)-aminocaproic acid-N-hydroxysuccinimide ester (biotin-7-NHS) by forming a stable amide bond. Non reacted biotin-7-NHS is separated on a Sephadex G-25 column.

The kit contains
1. Blocking reagent (green screw cap)
   One vial powder; it serves for saturation of the Sephadex G-25 columns to avoid unspecific binding of the protein to the column material thereby avoiding loss of yield.

2. Phosphate buffered saline (PBS) (blue screw cap)
   One vial powder; it serves for equilibration of the Sephadex G-25 columns after blocking, for dissolving, respectively diluting of the protein and for elution of the labeled protein from the column.

3. D-Biotinoyl-\(\epsilon\)-aminocaproic acid-N-hydroxysuccinimide ester (Biotin-7-NHS)
   Five reaction vials with 5 mg each, lyophilizate; biotin-7-NHS reacts with free amino groups of the proteins forming stable amide bonds (molecular weight: 454.5).

4. Dimethylsulfoxide (DMSO) (red screw cap)
   Two vials with 2.5 ml solution each; it serves as solvent for the biotin-7-NHS and if necessary for further dilutions.

5. Sephadex G-25 columns.
   The five columns serve for separation of non reacted biotin-7-NHS. The columns are preswollen, the volume of the column is 9.1 ml, the filling height is 5 cm. The maximal sample volume is 2.5 ml. The columns are fitted with a stopper on the top and the cap at the bottom and store the column at +2 to +8°C.

Procedure

Preparation of the columns
Not more than 10 mg protein (in a maximum of 2.5 ml) should be purified by each column. It should be taken care that the volume of the labeling mix does not exceed 2.5 ml since otherwise a correct separation can not be guaranteed.

• Fix the column with a clamp at a stand (if clamps and stand or adequate equipment is not available the column can also be held in a hand) and place an at least 100 ml containing beaker under the column.

• Open outlet of the column with scissors, remove the cap from the top of the column and let the content flow out. Add 5 ml blocking solution to the column and let run through. Rinse the column afterwards with 30 ml PBS solution in total (6 x 5 ml) and let it flow through. The column should not run dry! The Sephadex G-25 column is now ready to use. If the column is not immediately used, fix the stopper on the top and the cap at the bottom and store the column at +2 to +8°C.

Protein labeling with biotin-7-NHS
In the following chapter, five typical examples for protein labeling are described:

Note: Please be aware that exact experimental conditions have to be chosen and might be adjusted for your specific protein of interest (see Tab. 1).

The biotin labeling of 1 mg monoclonal antibody, of 1 mg polyclonal antibody, of 1 mg Fab fragments, of 1 mg F(ab’)\(_2\) fragment and of 1 mg TNF-\(\alpha\) or per-formed in a reaction volume of 1 ml is described.

1. Monoclonal antibody (M\(_2\); approx. 150 000):
   The molar reaction mixture is 1:10, i.e. 1 molecule of antibody is reacted with 10 molecules of biotin-7-NHS.
   
   • Dissolve 1 mg monoclonal antibody in 1 ml PBS. The monoclonal antibody should not be in a buffer containing primary amino groups (like Tris or glycine buffers or in a buffer containing sugars) otherwise it should be dialyzed against PBS prior to conjugation. Dilute 10 \(\mu\)l biotin-7-NHS solution (20 mg/ml) 1:10 with DMSO (10 \(\mu\)l biotin-7-NHS solution + 90 \(\mu\)l DMSO). From this dilution (2 mg/ml) add 15 \(\mu\)l (30 \(\mu\)g) to the antibody solution during stirring and incubate for 2 h at 15-25°C during gentle stirring.

   Reaction mixture for monoclonal antibodies

<table>
<thead>
<tr>
<th>molar ratio</th>
<th>antibody mg</th>
<th>antibody solution ml</th>
<th>biotin-7-NHS mg</th>
<th>biotin-7-NHS solution 20 mg/ml</th>
<th>biotin-7-NHS solution 2 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 10</td>
<td>1.0</td>
<td>1.0</td>
<td>0.030</td>
<td>–</td>
<td>15</td>
</tr>
</tbody>
</table>
2. Polyclonal antibody (Mr: approx. 150,000):
The molar reaction mix is 1:50, i.e. 1 molecule of antibody is reacted with 50 molecules of biotin-7-NHS.
- Dissolve 1 mg polyclonal antibody in 1 ml PBS. Add 76 μl biotin-7-NHS solution (20 mg/ml) to the antibody solution during stirring and incubate for 2 h at 15-25°C during gentle stirring.

Reaction mixture for polyclonal antibodies

<table>
<thead>
<tr>
<th>molar ratio</th>
<th>antibody mg</th>
<th>antibody solution ml</th>
<th>biotin-7-NHS mg</th>
<th>biotin-7-NHS solution 20 mg/ml μl</th>
<th>biotin-7-NHS solution 2 mg/ml μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 50</td>
<td>1.0</td>
<td>1.0</td>
<td>0.152</td>
<td>76</td>
<td>—</td>
</tr>
</tbody>
</table>

- Reaction mixture for Fab fragments

<table>
<thead>
<tr>
<th>molar ratio</th>
<th>antibody mg</th>
<th>antibody solution ml</th>
<th>biotin-7-NHS mg</th>
<th>biotin-7-NHS solution 20 mg/ml μl</th>
<th>biotin-7-NHS solution 2 mg/ml μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 10</td>
<td>1.0</td>
<td>1.0</td>
<td>0.045</td>
<td>4.5</td>
<td>—</td>
</tr>
</tbody>
</table>

3. F(ab')2 fragment (Mr: approx.100,000):
The molar reaction mix is 1:10, i.e. 1 molecule of antibody is reacted with 10 molecules of biotin-7-NHS.
- Dissolve 1 mg F(ab')2 fragment in 1 ml PBS. Dilute 10 μl biotin-7-NHS solution (20 mg/ml) : 1 : 10 with DMSO (10 μl biotin-7-NHS solution + 90 μl DMSO). From this dilution (2 mg/ml) add 22.5 μl (45 μg) to the antibody solution during stirring and incubate for 2 h at +15 to +25°C during gentle stirring.

Reaction mixture for F(ab')2 fragment

4. Fab fragments (Mr: approx. 50,000):
The molar reaction mix is 1 : 10, i.e. 1 molecule of antibody is reacted with 10 molecules of biotin-7-NHS.
- Dissolve 1 mg Fab fragments in 1 ml PBS. Add 4.5 μl biotin-7-NHS solution (20 mg/ml) to the antibody solution during stirring and incubate for 2 h at +15 to +25°C during gentle stirring.

Reaction mixture for Fab fragments

5. TNF-α (Mr: approx. 170,000):
The molar reaction mix is 1 : 5, i.e. 1 molecule of antibody is reacted with 5 molecules of biotin-7-NHS.
- Dissolve 1 mg TNF-α in 1 ml PBS. Add 6.7 μl biotin-7-NHS solution (20 mg/ml) to the antibody solution during stirring and incubate for 2 h at +15 to +25°C during gentle stirring.

Reaction mixture for TNF-α

Column chromatography
Remaining, non reacted biotin-7-NHS is separated by gel filtration on a prepared Sephadex G-25 column.
- Remove stopper and cap from the prepared column.
- Apply reaction mix (e.g. 1 ml + X μl biotin-7-NHS solution) to the column and let it flow through. Add PBS solution to a final volume of 2.5 ml (2.5 ml – ml reaction mix) to the column and let it flow through.
- Hold reaction vials in readiness to collect the samples and elute the labeled protein with 3.5 ml PBS solution. It is recommended to collect pools of 10 drops (0.5 ml). The labeled protein is present in the first 4 pools. The extinction at 280 nm of this 4 pools should be determined to be able to form a reasonable main pool. If a photometer is not available one should follow the guideline that 80% of the labeled protein is present in pool 2 and 3. A typical elution profile is shown in fig.1. After elution of the protein, the Sephadex G-25 column should be discarded.

Storage
The eluted conjugate is stable at +4°C. For longer storage we recommend the addition of 1% bovine serum albumine or 2% raffinose and the use of an antimicrobial agent.
Long term storage should be performed at −15 to −25°C.

Note
1. The above described reaction mixes are guidelines which result from experience; e.g., dependend on type and application of the protein the labeling might be optimizable by varying the reaction mix. The actual amount of biotin-7-NHS which should be applied can easily be quoted from table 1.
2. The labeling rate of the protein with biotin is not only dependend from the reaction stoichiometry, but also dependend from the reaction stoichiometry, but also from the concentration of the reaction partner: e.g., that in the reaction examples described above a higher labeling rate is to be expected when the reaction is performed in 100 μl than in 1 ml and than a lower labeling rate is to be expected, respectively, when the reaction is performed in 2.5 ml than in 1 ml. The molar reaction stoichiometry is each time the same.

Trouble shooting
Low labeling rate
- pH of conjugation mix was not well adjusted: The pH should be between pH 7 – 9.
- The protein to be coupled was in a buffer containing primary amino groups or sugars: Dialyze your protein against PBS.
- Biotin-7-NHS solution was not freshly prepared: Use only freshly prepared Biotin-7-NHS.
- Protein concentration was too low: The labeling rate of the protein with biotin is not only dependend from the reaction stoichiometry, but also from the concentration of the reaction partner: e.g., that in the reaction examples described above a higher labeling rate is to be expected when the reaction is performed in 100 μl than in 1 ml and than a lower labeling rate is to be expected, respectively, when the reaction is performed in 2.5 ml than in 1 ml. The molar reaction stoichiometry is each time the same.
- Proposed stoichiometry of reaction mix is not well suited: Try other protein to Biotin-7-NHS ratios.
- Turbid eluate
Centrifuge the solution at high speed prior to use.
Tab. 1: Molar reaction mixture

<table>
<thead>
<tr>
<th>$M_r$ protein</th>
<th>1 : 5</th>
<th>1 : 10</th>
<th>1 : 20</th>
<th>1 : 30</th>
<th>1 : 40</th>
<th>1 : 50</th>
<th>1 : 60</th>
<th>1 : 70</th>
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<th>1 : 100</th>
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<td>0.227</td>
<td>0.455</td>
<td>0.909</td>
<td>1.364</td>
<td>1.818</td>
<td>2.273</td>
<td>2.727</td>
<td>3.182</td>
<td>3.838</td>
<td>4.091</td>
<td>4.545</td>
<td>5.000</td>
</tr>
<tr>
<td>20 000</td>
<td>0.114</td>
<td>0.227</td>
<td>0.455</td>
<td>0.682</td>
<td>0.909</td>
<td>1.136</td>
<td>1.364</td>
<td>1.591</td>
<td>1.818</td>
<td>2.045</td>
<td>2.273</td>
<td>2.500</td>
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<tr>
<td>30 000</td>
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<td>0.152</td>
<td>0.303</td>
<td>0.488</td>
<td>0.806</td>
<td>0.758</td>
<td>0.909</td>
<td>1.081</td>
<td>1.212</td>
<td>1.383</td>
<td>1.818</td>
<td>1.667</td>
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<tr>
<td>40 000</td>
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<td>0.114</td>
<td>0.227</td>
<td>0.341</td>
<td>0.455</td>
<td>0.568</td>
<td>0.682</td>
<td>0.759</td>
<td>0.909</td>
<td>1.023</td>
<td>1.136</td>
<td>1.250</td>
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<td>50 000</td>
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<td>0.273</td>
<td>0.364</td>
<td>0.455</td>
<td>0.445</td>
<td>0.636</td>
<td>0.727</td>
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<td>1.000</td>
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<tr>
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<td>0.076</td>
<td>0.152</td>
<td>0.227</td>
<td>0.303</td>
<td>0.379</td>
<td>0.455</td>
<td>0.530</td>
<td>0.606</td>
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<td>0.065</td>
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<td>0.650</td>
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<tr>
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<td>0.511</td>
<td>0.568</td>
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<tr>
<td>90 000</td>
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<tr>
<td>100 000</td>
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<td>0.318</td>
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<td>0.409</td>
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<td>0.500</td>
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<tr>
<td>110 000</td>
<td>0.021</td>
<td>0.041</td>
<td>0.083</td>
<td>0.124</td>
<td>0.165</td>
<td>0.207</td>
<td>0.248</td>
<td>0.289</td>
<td>0.331</td>
<td>0.372</td>
<td>0.413</td>
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<tr>
<td>120 000</td>
<td>0.019</td>
<td>0.038</td>
<td>0.076</td>
<td>0.114</td>
<td>0.152</td>
<td>0.189</td>
<td>0.227</td>
<td>0.265</td>
<td>0.303</td>
<td>0.341</td>
<td>0.379</td>
<td>0.417</td>
</tr>
<tr>
<td>130 000</td>
<td>0.017</td>
<td>0.035</td>
<td>0.070</td>
<td>0.105</td>
<td>0.140</td>
<td>0.175</td>
<td>0.210</td>
<td>0.245</td>
<td>0.280</td>
<td>0.315</td>
<td>0.350</td>
<td>0.385</td>
</tr>
<tr>
<td>140 000</td>
<td>0.016</td>
<td>0.033</td>
<td>0.065</td>
<td>0.097</td>
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<td>0.162</td>
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<tr>
<td>150 000</td>
<td>0.015</td>
<td>0.030</td>
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<td>0.182</td>
<td>0.212</td>
<td>0.242</td>
<td>0.273</td>
<td>0.303</td>
<td>0.333</td>
</tr>
</tbody>
</table>

Note:
To calculate the volume of biotin-7-NHS solution needed, please choose the appropriate value (mg biotin-7-NHS/mg protein) from the table.

- If you want to use the 20 mg/ml biotin-7-NHS solution apply the following formula:
  value [mg biotin-7-NHS/mg protein] / 0.02 × actual protein amount [mg protein] = X μL biotin-7-NHS solution (20 mg/ml)

- If you want to use the 2 mg/ml biotin-7-NHS solution apply the following formula:
  value [mg biotin-7-NHS/mg protein] / 0.002 × actual protein amount [mg protein] = X μL biotin-7-NHS solution (2 mg/ml)

Changes to previous version
Editorial changes.

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