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

Biotin Protein Labeling Kit

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For labeling of proteins with biotin.

Cat. No. 11 418 165 001 1 kit
5 labeling reactions

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	green	Biotin Protein Labeling Kit, Blocking reagent	▪ Powder ▪ Saturates the Sephadex G-25 columns to avoid nonspecific binding of the protein to the column material, preventing a loss of yield.	1 vial
2	blue	Biotin Protein Labeling Kit, Phosphate buffered saline (PBS)	▪ Powder ▪ Equilibrates the Sephadex G-25 columns after blocking. ▪ To dissolve/dilute the protein and to elute the labeled protein from the column.	1 vial
3	–	Biotin Protein Labeling Kit, D-Biotinoyl-ε-aminocaproic acid-N-hydroxy-succinimide ester (Biotin-7-NHS)	▪ Lyophilized ▪ Reacts with free amino groups of the proteins forming stable amide bonds (molecular weight: 454.5).	5 vials, 5 mg each
4	red	Biotin Protein Labeling Kit, Dimethylsulfoxide (DMSO)	Solvent for the Biotin-7-NHS and for further dilutions.	2 vials, 2.5 ml each
5	–	Biotin Protein Labeling Kit, Sephadex G-25 column	▪ Pre-swollen, volume of column is 9.1 ml, filling height 5 cm. ▪ To separate non-reacted Biotin-7-NHS.	5 columns

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	green	Blocking reagent	Store at +2 to + 8°C.
2	blue	PBS	
3	–	Biotin-7-NHS	
4	red	Dimethylsulfoxide	
5	–	Sephadex G-25 column	Store at +2 to + 8°C. ⚠ ***Do not freeze.***

1.3. Application

Use the Biotin Protein Labeling Kit for the labeling of proteins with biotin in
- Immunohistochemistry
- Immunoblotting
- ELISA

2. How to Use this Product

2.1. Before you Begin

General Considerations

The procedures provided in section, **Protocols** are only guidelines. Depending on the type and application of the protein, the labeling can be optimized by varying the reaction mix. The correct amount of Biotin-7-NHS to be applied is shown in the table in section, **Protocols, Molar reaction mixture**.
The labeling rate of the protein with biotin depends on the reaction stoichiometry and on the concentration of the reaction partner. The labeling rate in the reaction examples described in section, **Protocols** can vary. For example, a higher labeling rate is to be expected when the reaction is performed in 100 µl rather than in 1 ml, and a lower labeling rate respectively when the reaction is performed in 2.5 ml rather than in 1 ml. The molar reaction stoichiometry remains the same.

Working Solution

Solution	Preparation	Storage and Stability	For use in...
Blocking solution	Dissolve the Blocking reagent (Vial 1) in 300 ml double-distilled water.	Store for 3 months at +2 to +8°C or in aliquots for 12 months at −15 to −25°C.	Saturation of Sephadex G-25 columns
PBS solution	Dissolve the PBS (Vial 2) in 1 l double-distilled water.		Antibody dilution
Biotin-7-NHS solution	Add 250 µl DMSO (Vial 4) to the Biotin-7-NHS (Vial 3); vortex several times. *The final concentration is 20 mg/ml.* ***For the labeling mix, the volume of the Biotin-7-NHS solution should not exceed 5% of the total reaction volume. For example, add 50 µl Biotin-7-NHS solution to 1 ml protein solution.***	***Prepare the solution directly before labeling.*** Store for 1 week at +2 to +8°C.	Antibody solution

2.2. Protocols

Preparation of the columns

⚠ ***Do not purify more than 10 mg protein in a maximum of 2.5 ml per column.***
⚠ ***Ensure that the volume of the labeling mix does not exceed 2.5 ml for a correct separation.***

1. Fix the column with a clamp onto a stand and place into a beaker that holds at least 100 ml below the column.
   ℹ *Alternatively, if the clamp and stand are not available, hold the column by hand.*

2. Open outlet of the column with scissors.

3. Remove the cap from the top of the column and let the contents run out.

4. Add 5 ml Blocking solution.

5. Rinse the column 6 times with 5 ml PBS solution.
   ⚠ ***Do not let the column run dry.***
   ℹ *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.*
   ⚠ ***Do not freeze the columns.***

Protein labeling with Biotin-7-NHS

ℹ *Experimental conditions may need to be adjusted for your specific protein of interest, see table in* **Molar reaction mixture**.

Monoclonal antibody (Mr: approximately 150,000)

The molar reaction mixture is 1:10, for example, 1 molecule of antibody for 10 molecules of Biotin-7-NHS.

1. Dissolve 1 mg monoclonal antibody in 1 ml PBS.
   ⚠ ***Do not use buffers containing primary amino groups, such as Tris, glycine buffers, or buffers containing sugars, as the antibody dialyzes against PBS prior to conjugation.***

2. Dilute 10 µl Biotin-7-NHS solution (20 mg/ml) 1:10 with DMSO (10 µl Biotin-7-NHS solution + 90 µl DMSO).

3. Add 15 µl (30 µg) of the dilution from Step 2 (2 mg/ml) to the antibody solution (Step 1) under stirring.

4. Incubate for 2 hours at +15 to +25°C under gentle stirring.

Reaction mixture for monoclonal antibodies

Molar ratio	Antibody [mg]	Antibody solution [ml]	Biotin-7-NHS [mg]	Biotin-7-NHS solution 20 mg/ml [µl]	Biotin-7-NHS solution 2 mg/ml [µl]
1:10	1.0	1.0	0.030	–	15

Polyclonal antibody (Mr: approximately 150,000)

The molar reaction mix is 1:50, for example, 1 molecule of antibody for 50 molecules of Biotin-7-NHS.

1. Dissolve 1 mg polyclonal antibody in 1 ml PBS.
2. Add 7.6 µl Biotin-7-NHS solution (20 mg/ml) to the antibody solution (Step 1) under stirring.
3. Incubate for 2 hours at +15 to +25°C under gentle stirring.

Reaction mixture for polyclonal antibodies

Molar ratio	Antibody [mg]	Antibody solution [ml]	Biotin-7-NHS [mg]	Biotin-7-NHS solution 20 mg/ml [µl]	Biotin-7-NHS solution 2 mg/ml [µl]
1:50	1.0	1.0	0.152	7.6	–

F(ab′)$_2$ fragment (Mr: approximately 100,000)

The molar reaction mix is 1:10, for example, 1 molecule of antibody for 10 molecules of Biotin-7-NHS.

1. Dissolve 1 mg F(ab′)$_2$ fragment in 1 ml PBS.
2. Dilute 10 µl Biotin-7-NHS solution (20 mg/ml) 1:10 with DMSO (10 µl Biotin-7-NHS solution + 90 µl DMSO).
3. From the dilution from Step 2 (2 mg/ml), add 22.5 µl (45 µg) to the antibody solution (Step 1) under stirring.
4. Incubate for 2 hours at +15 to +25°C during gentle stirring.

Reaction mixture for F(ab′)$_2$ fragment

Molar ratio	Antibody [mg]	Antibody solution [ml]	Biotin-7-NHS [mg]	Biotin-7-NHS solution 20 mg/ml [µl]	Biotin-7-NHS solution 2 mg/ml [µl]
1:10	1.0	1.0	0.045	–	22.5

Fab fragments (Mr: approximately 50,000)

The molar reaction mix is 1:10, for example, 1 molecule of antibody for 10 molecules of Biotin-7-NHS.

1. Dissolve 1 mg Fab fragments in 1 ml PBS.
2. Add 4.5 µl Biotin-7-NHS solution (20 mg/ml) to the antibody solution (Step 1) under stirring.
3. Incubate for 2 hours at +15 to +25°C under gentle stirring.

Reaction mixture for Fab fragments

Molar ratio	Antibody [mg]	Antibody solution [ml]	Biotin-7-NHS [mg]	Biotin-7-NHS solution 20 mg/ml [µl]	Biotin-7-NHS solution 2 mg/ml [µl]
1:10	1.0	1.0	0.091	4.5	–

TNF-α (Mr: approximately 17,000)

The molar reaction mix is 1:5, for example, 1 molecule of antibody for 5 molecules of Biotin-7-NHS.

1. Dissolve 1 mg TNF-α in 1 ml PBS.
2. Add 6.7 µl Biotin-7-NHS solution (20 mg/ml) to the antibody solution under (Step 1) under stirring.
3. Incubate for 2 hours at +15 to +25°C under gentle stirring.

Reaction mixture for TNF-α

Molar ratio	TNF-α [mg]	TNF-α solution [ml]	Biotin-7-NHS [mg]	Biotin-7-NHS solution 20 mg/ml [µl]	Biotin-7-NHS solution 2 mg/ml [µl]
1:5	1.0	1.0	0.133	6.7	–

Column chromatography

The remaining nonreacted Biotin-7-NHS is separated by gel filtration on a prepared Sephadex G-25 column.

1. Remove stopper and cap from the prepared column.

2. Apply reaction mix, such as 1 ml + X µl Biotin-7-NHS solution to the column and let it flow through.

3. Add PBS solution to a final volume of 2.5 ml (2.5 ml minus respective ml of reaction mix) to the column and let it flow through.

4. Collect the samples in reaction vials and elute the labeled protein with 3.5 ml PBS solution.
   – For best results, collect pools of 10 drops (0.5 ml).

5. The labeled protein is present in the first 4 pools.
   – Determine the extinction at 280 nm of these 4 pools to achieve a sufficient main pool.
   If a photometer is not available, follow the general guideline that 80% of the labeled protein is present in pools 2 and 3.

6. After elution of the protein, discard the Sephadex G-25 column.

7. The eluted conjugate is stable at +4°C.
   – For longer storage, add 1% BSA or 2% raffinose and an antimicrobiological agent; store at −15 to −25°C.

An elution profile is shown in Figure 1.

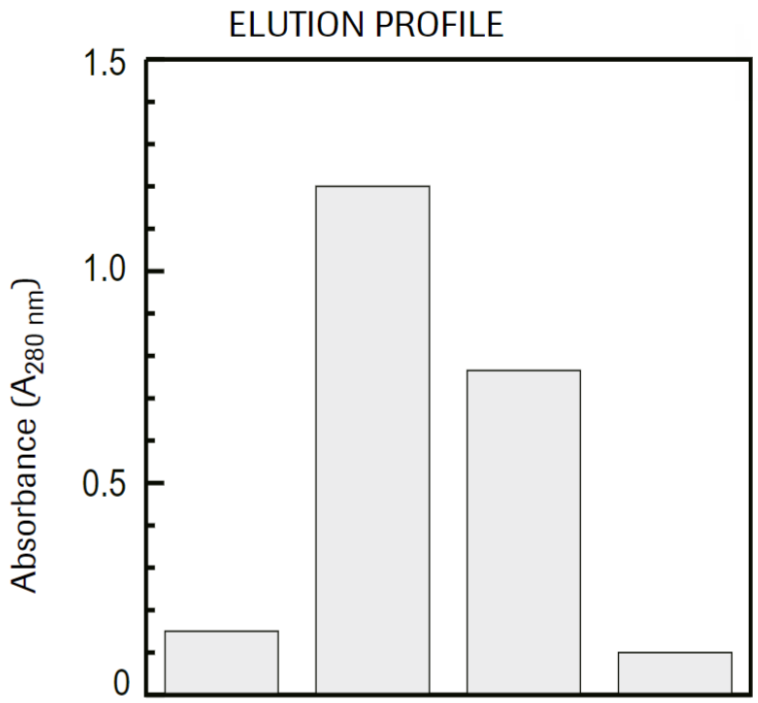

Fig. 1: Fractions 1 to 4: 10 drops per fraction (10 drops are approximately 0.5 ml).

Molar reaction mixture

	Molar reaction mixture (molecules protein/molecules Biotin-7-NHS)											
Mr protein	1:5	1:10	1:20	1:30	1:40	1:50	1:60	1:70	1:80	1:90	1:100	1:110
	mg Biotin-7-NHS/mg protein											
10,000	0.227	0.455	0.909	1.364	1.818	2.273	2.727	3.182	3.838	4.091	4.545	5.000
20,000	0.114	0.277	0.455	0.682	0.909	1.136	1.364	1.591	1.818	2.045	2.273	2.500
30,000	0.076	0.152	0.303	0.488	0.806	0.758	0.909	1.081	1.212	1.383	1.818	1.667
40,000	0.057	0.114	0.227	0.341	0.455	0.568	0.682	0.759	0.909	1.023	1.136	1.250
50,000	0.045	0.091	0.182	0.273	0.364	0.455	0.445	0.636	0.727	0.818	0.909	1.000
60,000	0.038	0.076	0.152	0.227	0.303	0.379	0.455	0.530	0.606	0.682	0.758	0.833
70,000	0.032	0.065	0.130	0.195	0.260	0.325	0.390	0.455	0.519	0.584	0.650	0.714
80,000	0.028	0.057	0.114	0.170	0.227	0.284	0.341	0.398	0.455	0.511	0.568	0.625
90,000	0.025	0.051	0.101	0.152	0.202	0.252	0.303	0.354	0.404	0.455	0.505	0.556
100,000	0.023	0.045	0.091	0.136	0.182	0.227	0.273	0.318	0.364	0.409	0.455	0.500
110,000	0.021	0.041	0.083	0.124	0.165	0.207	0.248	0.289	0.331	0.372	0.413	0.455
120,000	0.019	0.038	0.076	0.114	0.152	0.189	0.227	0.265	0.303	0.341	0.379	0.417
130,000	0.017	0.035	0.070	0.105	0.140	0.175	0.210	0.245	0.280	0.315	0.350	0.385
140,000	0.016	0.033	0.065	0.097	0.130	0.162	0.195	0.227	0.260	0.292	0.325	0.357
150,000	0.015	0.030	0.061	0.091	0.121	0.152	0.182	0.212	0.242	0.273	0.303	0.333

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

Amount of solution [mg/ml]	Formula
20	value (mg Biotin-7-NHS/mg protein)/0.02 × actual protein amount (mg protein) = X µl Biotin-7-NHS solution (20 mg/ml)
2	value (mg Biotin-7-NHS/mg protein)/0.002 × actual protein amount (mg protein) = X µl Biotin-7-NHS solution (2 mg/ml)

3. Troubleshooting

Observation	Possible cause	Recommendation
Low labeling rate.	Wrong pH value.	Readjust the pH value of the conjugation mix to pH 7 to 9.
	Buffer contained primary amino groups or sugars.	Dialyze protein against PBS.
	Biotin-7-NHS solution too old.	Prepare a fresh solution.
	Protein concentration too low.	Use a protein concentration of 5 to 15 mg/ml for optimal labeling rates. ⚠ ***If the total amount of protein exceeds 10 mg, separation must be performed in more than one column.***
	Wrong stoichiometry of reaction mix.	Try other protein to Biotin-7-NHS ratios.
Turbid eluate present.		Centrifuge the solution at high speed prior to use.

4. Additional Information on this Product

4.1. Test Principle

Free amino groups of the protein to be labeled react with D-biotinoyl-ε-aminocaproic acid-N-hydroxysuccinimide ester (Biotin-7-NHS) by forming a stable amide bond. Nonreacted Biotin-7-NHS is separated on a Sephadex G-25 column.

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Trademarks

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

5.4. License Disclaimer

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

5.5. Regulatory Disclaimer

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

5.6. Safety Data Sheet

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

5.7. 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.

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