Biotin and an enzyme-conjugated streptavidin are used for highly sensitive detection. Biotin-PEAC₅-maleimide is one of the sulphydryl reactive biotins used for biotin labeling of proteins such as antibodies which is modified by reducing agent. Biotin-PEAC₅-maleimide reacts with a sulphydryl group under the physiological conditions. Though Biotin-PEAC₅-maleimide is slightly water-soluble, an organic solvent such as DMSO or DMF is required to prepare a stock solution. The stock solution can be mixed with buffers containing materials to be biotinylated. Biotinylation does not cause serious activity loss of proteins due to the molecular size of biotin. Since Biotin-PEAC₅ has a longer spacer than biotin, the active site of the protein is not blocked when enzyme-conjugated streptavidin is bound at the biotinylated site.

### Chemical name

**N-6-(Biotinylamino)hexanoyl-N’-[2-(N-maleimide)ethyl]piperazone, hydrochloride**

**Appearance**

white or slightly yellow powder

**Purity**

>90% by HPLC

**Molecular Weight**

585.16, C₂₆H₄₁ClN₆O₅S

**CAS Number**

[   -   ]

**Storage condition**

Freezer (-20 °C)

**Disposal method**

Described in MSDS

### Required Equipment and Materials

- DMSO or DMF
- Reaction buffer
- 10 µl, 200 µl, and 1000 µl adjustable pipettes
- Microtubes or centrifuge tubes
- Incubator, 30 - 37 °C

### Purification/Isolation

- Dialysis tube (10,000 MW) or Shephadex gel column (PD10 or NAP5)
- PBS or HEPES buffer

### Analysis/Isolation

- HPLC
- TLC

### General Protocol for Protein

1. Weigh the necessary amount of Biotin-PEAC₅-maleimide for a conjugation reaction.
2. Dissolve the Biotin-PEAC₅-maleimide with DMSO or DMF.
3. Prepare a protein solution with an appropriate buffer.
4. Add the Biotin-PEAC₅-maleimide solution to the protein solution and mix by pipetting.
5. Incubate the tube at 30 - 37 °C for 30 min.
6. Use a Sephadex gel column (PD10 or NAP5) or a dialysis tube to remove excess Biotin-PEAC₅-maleimide and other small molecules.
7. Concentrate the gel filtrate or dialyzed solution with a membrane filter.
8. Store the conjugate at 0-5 °C.

### Precautions

Use freshly prepared Biotin-PEAC₅-maleimide solution for the conjugation reaction.

Thiol compounds interfere with the Biotin-PEAC₅-maleimide reaction. If the buffer solution of the macromolecule contains such compounds, change the buffer to remove those molecules.

**a)** The necessary amount of Biotin-PEAC₅-maleimide can be calculated with the following equation. The amount of Biotin-PEAC₅-maleimide will be 40 times the molar equivalent of the protein.

\[
\text{Biotin-PEAC}_{\text{s}}-\text{maleimide (mg)} = 22 \times \text{protein (\(\mu\text{mol}\) )}
\]

The following chart indicates how the conditions affect the number of maleimide groups introduced into the protein. If it is necessary to control the number of biotin molecules per protein, modify the reaction condition.

<table>
<thead>
<tr>
<th>Condition</th>
<th>more biotin</th>
<th>less biotin</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin-PEAC₅-maleimide</td>
<td>increase</td>
<td>decrease</td>
<td>1 - 110*</td>
</tr>
<tr>
<td>Reaction pH</td>
<td>increase</td>
<td>decrease</td>
<td>6 - 7.8</td>
</tr>
<tr>
<td>Reaction temperature</td>
<td>-</td>
<td>decrease</td>
<td>4 - 37 °C</td>
</tr>
<tr>
<td>Reaction time</td>
<td>increase</td>
<td>decrease</td>
<td>10 min - 2 hours (37 °C)</td>
</tr>
</tbody>
</table>

* the number of the equation above

**b)** The volume of the DMSO or DMF is about 0.5 ml per 1 mg Biotin-PEAC₅-maleimide. DMSO or DMF solution of Biotin-PEAC₅-maleimide is stable for several hours at ambient temperature.

**c)** Use a neutral pH buffer, such as pH 7.4 PBS or HEPES buffer. The volume of the buffer is about 1 ml per 1 mg protein.

**d)** Use a neutral buffer for a gel column filtration or a dialysis. Alternatively, a membrane filtration tube with an appropriate pore size can be used if the protein does not aggregate on the membrane. Wash the conjugate with a neutral buffer at least three times.

**e)** An appropriate concentration of the maleimido-protein is about 250 ul per 1 mg protein. Excessively high concentration may cause aggregation of proteins.
The average number of biotin molecules per IgG is about 5 to 8. If you need to determine the precise number of biotin molecules per protein, use HABA assay. The following is a HABA assay protocol.

**Reagent solution:**
- 200 µM HABA (4-hydroxyazobenzene-2-carboxylic acid) solution prepared with PBS, pH 7.4 .......................... 1 ml
- 25 M biotin prepared with a mixed solution (2 volumes of PBS, pH 7.4 + 1 volume of WS buffer) ................. 200 l
- Prepare various concentration solutions (12.5 M, 6.25 M, 3.13 M, 1.56 M) with serial dilution .............. 200 l each

**Protocol**
1. Mix HABA solution and avidin solution in a plastic tube.
2. Add 100 l of the HABA-avidin solution to 15 wells of a 96-well plate for multiple assays (n=3).
3. Add 50 l of biotin solution (12.5 M, 6.25 M, 3.13 M, and 1.56 M) to 3 wells each and 50 l of diluted sample solution to the rest of the 3 wells.
4. Read the O.D. at 405 nm with a reference at 492 nm and prepare a calibration curve using the O.D. of various concentrations of biotin solution. Read the O.D. at 280 nm to determine the protein concentration. (e.g. molar absorbity of IgG at 280 nm: 216,000).
5. Determine the concentration of biotin in the sample solution and calculate the number of biotin molecules per protein.

**Abbreviations**
- DMF: dimethylformamide
- HPLC: high performance liquid chromatography
- PBS: phosphate buffered saline
- TLC: thin layer chromatography
- NHS: N-hydroxysuccinimide
- Sephadex: trademark of GE HealthCare
- PD10, NAP5: products of GE HealthCare

**References**

Since this technical manual does not cover all conditions, please contact our customer services for more information at info@dojindo.com or click on “Technical Questions” on our web site (www.dojindo.com) and fill out the form with your specific question.