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## 1. Description

<b>Components</b>	1 strip of 8 reaction wells, each containing lyophilized Biotinylation Mix.
<b>Capacity</b>	For 8 labeling reactions of up to 10 µg of antibody each.
<b>Product format</b>	The labeling agent is supplied as a lyophilized powder and is optimally apportioned for the labeling of up to 10 µg of antibody per well. Each well can be cut from the strip for individual use or multiple antibodies can be labeled simultaneously.
<b>Storage</b>	Store at -20 °C. Warm kit to room temperature before use. The expiration date is indicated on the bag label.

### 1.1 Principle of the One-step Antibody Biotinylation Kit

The One-step Antibody Biotinylation Kit is optimized for the rapid and easy biotinylation of antibodies. The lyophilized labeling agent allows the simple resuspension and incubation with the antibody of choice, after which the biotinylated antibody can be directly used. Each well contains enough label for the optimal biotinylation of 10 µg of antibody.

The antibody is simply added to the lyophilized biotinylation mix and incubated at room temperature. The labeled antibody can be directly used in downstream applications, no post-conjugation purification is necessary.

### 1.2 Background information

The One-step Antibody Biotinylation Kit has been developed for the biotinylation of monoclonal antibodies for use in magnetic cell separation (with MACS® Technology) as well as fluorescent cell analysis.

Once biotinylated, antibodies can be directly used to tag target cells. Subsequently, target cells can either be magnetically labeled using Anti-Biotin MicroBeads (# 130-090-485) or fluorescently stained with a FITC-, PE-, or APC-conjugated anti-biotin antibody. After labeling with Anti-Biotin MicroBeads, target cells can be enriched according to the protocol in the Anti-Biotin MicroBeads data sheet.

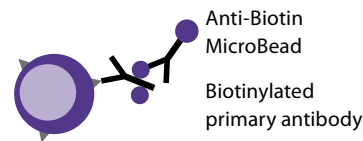


Figure 1: Immunomagnetic labeling principle.

### 1.3 Applications

- Biotinylation of monoclonal antibodies.

### 1.4 Reagent requirements

- **Antibody:** The antibody to be biotinylated must be purified from azide, serum components, and other NH<sub>2</sub>-containing molecules prior to biotinylation. The antibody should be prepared at a concentration of 100 µg/mL in PBS. A maximum of 10 µg of antibody can be labeled per well.
- (Optional) Anti-Biotin MicroBeads (# 130-090-485).
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), Anti-Biotin-APC (# 130-090-856), Anti-Biotin-VioBlue® (# 130-094-669), or Anti-Biotin-PerCP (# 130-094-974) for use as a secondary antibody reagent.

## 2. General protocol for immunofluorescent staining

▲ The following protocol is for the biotinylation of 100  $\mu\text{L}$  of a single antibody adjusted to a concentration of 100  $\mu\text{g}/\text{mL}$  in PBS. As described above, the antibody must be free of azide, serum components, and other  $\text{NH}_2$ -containing molecules.

1. Cut off and thaw the required number of wells from the strip. Wells must be warmed to room temperature before use.
2. Add 100  $\mu\text{L}$  of the antibody to be biotinylated to the well. Resuspend the lyophilized powder fully by mixing thoroughly.
3. Incubate the mixture at a controlled room temperature (18–25  $^{\circ}\text{C}$ ) for 24 h.
4. After incubation, the antibody is ready for direct use in cell labeling or other downstream applications.

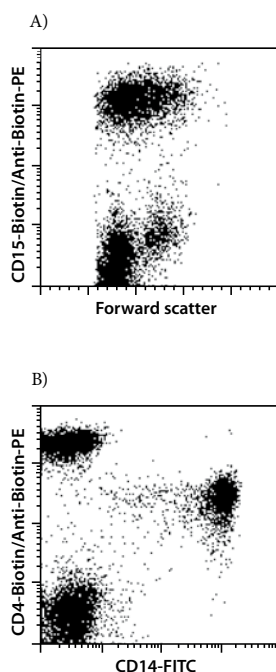
▲ **Note:** Optimal staining concentrations must be determined

▲ **Note:** Store conjugated antibodies at 2–8  $^{\circ}\text{C}$ . For long-term storage, addition of preservative is recommended.

▲ **Note:** Avoid use of streptavidin or Streptavidin MicroBeads as a second step reagent as residual free biotin in the labeling mixture might cause increased background staining. Free biotin is not detected by Anti-Biotin MicroBeads and -antibodies

## 3. Examples of immunofluorescent staining with biotinylated antibodies

With the One-step Antibody Biotinylation Kit, 10  $\mu\text{g}$  of unconjugated CD15 and CD4 antibodies were biotinylated as described. Human peripheral blood mononuclear cells (PBMCs) were then stained with the biotinylated antibodies as well as Anti-Biotin-PE (# 130-090-756) (A, B). Cells labeled with biotinylated CD4 (VIT4) were also stained with CD14-FITC (B). Cells were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

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