

Contents

1. Description
 - 1.1 Principle of the One-step Antibody Biotinylation Kit
 - 1.2 Background information
 - 1.3 Applications
 - 1.4 Reagent requirements
2. Protocol
3. Examples of immunofluorescent staining with biotinylated antibodies

1. Description

This product is for research use only.

Components	1 strip of 8 reaction wells, each containing lyophilized Biotinylation Mix.
Capacity	For 8 labeling reactions of up to 10 µg of antibody each.
Product format	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.
Storage	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.

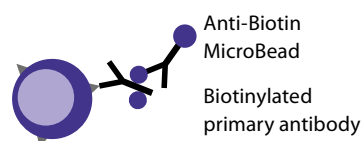


Figure 1: Immunomagnetic labeling principle.

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 NH₂-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 μ L of a single antibody adjusted to a concentration of 100 μ g/mL in PBS. As described above, the antibody must be free of azide, serum components, and other 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 μ 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 $^{\circ}$ C) for 24 h.
4. After incubation, the antibody is ready for direct use in cell labeling or other downstream applications.

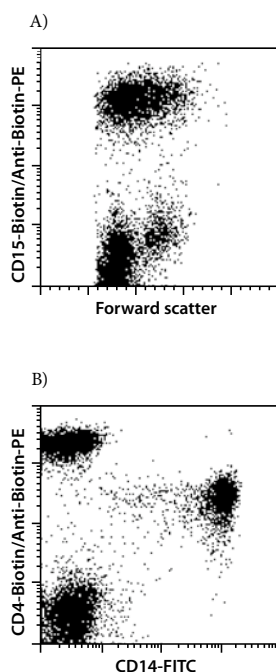
▲ **Note:** Optimal staining concentrations must be determined

▲ **Note:** Store conjugated antibodies at 2–8 $^{\circ}$ 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 μ 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.



Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

MACS, the Miltenyi logo, and VioBlue are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.

Copyright © 2021 Miltenyi Biotec and/or its affiliates. All rights reserved.