

Product Information

Biotin SE Protein Labeling Kit

Catalog Number: 92224

Size: 3 labeling reactions (up to 1 mg protein each)

Storage: 4°C

Storage and Handling: Stable for at least 3 months from date of receipt when stored as recommended. Stable for at least 6 months from date of receipt if the biotin SE vials (component A) are stored separately at -20°C.

Kit Components

Material	Quantity
Biotin SE (Component A)	3 vials
DMSO, anhydrous (#99953)	1 X 150 uL
Sodium bicarbonate solution, 1 M, pH 8.3 (#99954)	1 X 1 mL
1X PBS, pH 7.4 (#99955)	1 X 50 mL
Ultrafiltration vial, 10K MWCO (#99956)	3 vials
Reaction vial (#99957)	3 vials
Storage vial (#99958)	3 vials

Product Description

The Biotin SE Protein Labeling Kit provides a convenient way to label antibodies or other purified proteins with biotin. The kit contains biotin succinimidyl ester (biotin SE) for covalent labeling of lysine residues in proteins and other free amine groups, plus everything else you need for carrying out the labeling reaction and purifying the labeled product. The reactive biotin is supplied in three vials, each of which is sufficient for labeling 1 mg of an IgG antibody or other protein. Following the labeling reaction, unconjugated biotin is conveniently and rapidly removed by ultrafiltration using the ultrafiltration vials provided.

Protocol for Labeling IgG antibodies

The protocol below is for labeling 1 mg of an IgG antibody. The procedure may be scaled up or down for any amount of protein as long as the ratios of the reagents are maintained. Note: the kits are not recommended for labeling IgM antibodies, because the alkaline pH at which labeling is carried out may cause denaturation of IgM. The kit can be used to label non-IgG proteins. The ratio of dye stock solution to protein amount may require optimization for different proteins. Depending on the protein molecular weight, the dye removal method may need to be modified; to remove free dye by ultrafiltration, the protein should be at least 3X larger than the molecular weight cut-off of the ultrafiltration membrane.

Note: Warm all reagents to room temperature before use.

1. Prepare the Antibody for Labeling

If the antibody is already in solution at ≥ 1 mg/mL in PBS or a similar buffer free of any amine-containing chemicals or preservatives, such as Tris, ammonium or free amino acids (such as glycine), proceed to step 2. However, if any amine-containing chemical is present, perform ultrafiltration using the filtration vial (#99956) provided in the kit. Sodium azide does not affect the labeling.

The ultrafiltration column membrane has a molecular weight cut-off of 10,000. Therefore, molecules smaller than 10 kDa will flow through the membrane, and molecules larger than 10 kDa, including IgG antibodies, will be retained on the upper surface of the membrane (Figure 1). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody.

Note: For ultrafiltration of proteins with molecular weight of 10-30 kDa, we recommend using ultrafiltration vials with 3 kDa molecular weight cut-off (catalog no. 22018).

Ultrafiltration Vial Capacities:
Maximum Sample Volume: 500 μ L
Final Concentrate Volume: 15 μ L
Filtrate Receiver Volume: 500 μ L
Hold-up Volume (Membrane/Support): < 5 μ L

Ultrafiltration Protocol

- Add an appropriate amount of antibody to the membrane of the ultrafiltration vial, being careful not to touch the membrane. Spin the solution at 14,000 x g in a microcentrifuge for one minute. Check to see how much liquid has filtered into the filtrate collection tube (lower chamber). Repeat the centrifugation until all of the liquid has filtered into the collection tube. Discard the liquid in the collection tube.
 - For antibody concentration, proceed to Step 3. For clean-up, add an equal volume of 1X PBS to the membrane. Spin the vial at 14,000 xg until the liquid has filtered into the filtrate receiving tube.
 - Add 0.9 mL PBS to the membrane. Carefully pipet the PBS up and down over the upper surface of the membrane to recover and resuspend the antibody.
 - Transfer the recovered antibody solution to a fresh microcentrifuge tube.
 - Important:** Save the ultrafiltration vial to reuse in the antibody purification step (Step 3). Ultrafiltration vials also can be purchased separately (catalog no. 22004).
- Carry Out the Labeling Reaction
 - Add 100 μ L of 1M sodium bicarbonate pH 8.3 (#99954) to the 900 antibody solution from step 1.4. If you did not perform ultrafiltration, adjust the antibody concentration to 1-2 mg/mL in 1X PBS and add 1/10 volume of 1M sodium bicarbonate pH 8.3.

Note: Labeling efficiency varies with antibody concentration. In general, the higher the antibody concentration, the higher the labeling efficiency. This protocol is optimized for 1-2 mg/mL antibody concentration.
 - Allow a vial of biotin SE to warm up to room temperature, and then add 25 μ L anhydrous DMSO (#99953) to the biotin SE vial. Vortex to dissolve the biotin SE and then centrifuge briefly to collect the solution at bottom of the vial.
 - Transfer the biotin SE stock solution from Step 2.2 to the antibody solution prepared in Step 2.1 and mix well. Incubate the reaction for 1 hour at room temperature with gentle rocking.

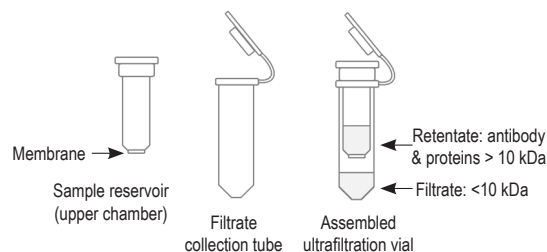


Figure 1. Ultrafiltration vial components.

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3. Purify the Labeled Antibody

See section 1 for a description of the ultrafiltration vial. For ultrafiltration of proteins with molecular weight of 10-30 kDa, we recommend using ultrafiltration vials with 3 kDa molecular weight cut-off (catalog no. 22018).

3.1 Transfer up to 0.5 mL of the reaction solution from Step 2.3 to the upper chamber of a filtration vial (#99956). Centrifuge the vial at 14,000 xg until nearly all of the liquid is in the collection tube below (5-10 min). Empty the collection tube, which contains unconjugated free biotin.

Caution! Avoid touching the membrane of the filtration vial during liquid transfer using a pipet. Any damage to the membrane may result in loss of antibody.

3.2 Repeat step 3.1 until all of the antibody/biotin solution has been centrifuged. A small amount of 1X PBS may be used to rinse the reaction vial and complete the solution transfer.

3.3 Add up to 0.5 mL 1X PBS (#99955) to the upper chamber of the filtration vial, which now contains the labeled antibody. Flick the vial gently to fully dissolve the labeled antibody. Centrifuge again to filter the antibody solution.

3.4 Repeat Step 3.3 two more times.

3.5 Add an appropriate amount of 1X PBS to reach the desired antibody concentration and store the solution in the storage vial (#99958) provided. Typical recovery is 80-90%.

4. Storage of Labeled Antibody

5.1 Store the labeled antibody at 4 °C.

Note: Long term storage of antibodies at less than 0.1 mg/mL may result in adsorption of antibody to plastic vials and degradation of antibody.

Related Products

Catalog #	Product Name	Unit Size
22004	Ultrafiltration vial, 10K MWCO	5 per pack
22018	Ultrafiltration vial, 3K MWCO	5 per pack
92286	Mix-n-Stain™ Biotin Antibody Labeling Kit, 5-20 ug	1 labeling
92266	Mix-n-Stain™ Biotin Antibody Labeling Kit, 20-50 ug	1 labeling
92244	Mix-n-Stain™ Biotin Antibody Labeling Kit, 50-100 ug	1 labeling
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
23003	EverBrite™ Hardset Mounting Medium	10 mL
23004	EverBrite™ Hardset Mounting Medium with DAPI	10 mL
23005	CoverGrip™ Coverslip Sealant	15 mL
22005	Mini Super ^{HT} Pap Pen 2.5 mm tip, ~400 uses	1 pen
22006	Super ^{HT} Pap Pen 4 mm tip, ~800 uses	1 pen

Streptavidin and anti-biotin antibody conjugates

Catalog #	Product Name	Unit Size
29031	Streptavidin, CF350 conjugate	1 mg
29032	Streptavidin, CF405S conjugate	1 mg
29033	Streptavidin, CF405M conjugate	1 mg
29034	Streptavidin, CF488A conjugate	1 mg
29030	Streptavidin, CF532 conjugate	1 mg
29038	Streptavidin, CF555 conjugate	1 mg
29035	Streptavidin, CF568 conjugate	1 mg
29036	Streptavidin, CF594 conjugate	1 mg
29037	Streptavidin, CF633 conjugate	1 mg
29041	Streptavidin, CF640R conjugate	1 mg
29039	Streptavidin, CF647 conjugate	1 mg
29040	Streptavidin, CF660R conjugate	1 mg
29044-200uL	Streptavidin, R-PE conjugate, 0.5 mg/mL	200 uL
29044-1mL	Streptavidin, R-PE conjugate, 0.5 mg/mL	1 mL
29048-200uL	Streptavidin, APC crosslinked conjugate, 0.5 mg/ml	200 uL
29048-1mL	Streptavidin, APC crosslinked conjugate, 0.5 mg/ml	1 mL
29049	Streptavidin, HRP conjugate	1 mg
20203	CF405S monoclonal mouse anti-biotin IgG, 2 mg/mL	0.25 mL
20203-1	CF405S monoclonal mouse anti-biotin IgG, 2 mg/mL	50 uL
20204	CF488A monoclonal mouse anti-biotin IgG, 2 mg/mL	0.25 mL
20204-1	CF488A monoclonal mouse anti-biotin IgG, 2 mg/mL	50 uL
20205	CF594 monoclonal mouse anti-biotin IgG, 2 mg/mL	0.25 mL
20205-1	CF594 monoclonal mouse anti-biotin IgG, 2 mg/mL	50 uL
20206	CF633 monoclonal mouse anti-biotin IgG, 2 mg/mL	0.25 mL
20206-1	CF633 monoclonal mouse anti-biotin IgG, 2 mg/mL	50 uL
20207	CF640R monoclonal mouse anti-biotin IgG, 2 mg/mL	0.25 mL
20207-1	CF640R monoclonal mouse anti-biotin IgG, 2 mg/mL	50 uL

Please visit www.biotium.com to view our full selection of biotinylation reagents, fluorescent CF™ reactive dyes, Mix-n-Stain™ antibody labeling kits, VivoBrite™ antibody labeling kits for small animal imaging, a wide selection of CF™ dye conjugates, and other innovative products for genomics, proteomics, and cell biology research.

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