Product Information

Super-n-Stain™ Antibody Labeling Kits

Catalog Number: S6011

Product Size: S, M, L

Contents:

Component	S（5-20 µg）	M（20-50 µg）	L（50-100 µg）
A. Dye vial	1 vial	1 vial	1 vial
B. Super-n-Stain™ reaction buffer, 10×	1 vial of 15 µL	1 vial of 15 µL	1 vial of 30 µL
C. Super-n-Stain™ antibody storage buffer	1 vial of 60 µL	1 vial of 150 µL	1 vial of 300 µL
D. Ultrafiltration vial (MWCO=10K)	1 each	1 each	1 each

Storage

Store at -20℃ and protect from light. When stored as directed, product is stable for at least 3 months.

Description

Super-n-Stain™ antibody labeling kits contain everything you need to rapidly label an antibody with YF® dyes or biotin. Simply mix your antibody with the reaction buffer and pre-measured dye provided, followed by a brief incubation. Super-n-Stain™ Dye is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. The antibody will be labeled with an average of 4-6 dye/label molecules per antibody molecule. Super-n-Stain™ labeling is covalent, so labeled antibodies can be used for multiplex staining without transfer of dyes/labels between antibodies.

Super-n-Stain™ labeling can tolerate sodium azide and glycine, as well as low levels of glycerol and Tris. A microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove incompatible small molecule buffer components.

A modified protocol is provided for labeling antibodies with more than 4-fold excess BSA or gelatin. Select the kit size based on the amount of IgG you want to be labeled. The optimized labeling procedure is suitable for stabilizing IgG samples with excess protein or ascites. The modified labeling protocol can also be used to label samples with IgG content lower than the lowest range, and the total protein content can reach the labeling range of the kit by adding stabilizer protein. The modified procedure is not recommended for labeling the supernatant of natural antiserum or hybridoma cell lines because the antibody content of these samples is very low relative to the total protein.

When performing direct immunofluorescence with a fluorescently-labeled antibody, you may need to use a higher concentration of antibody to achieve similar staining intensity compared to indirect immunofluorescence detection using unlabeled primary plus labeled secondary antibody. In our internal testing, indirect immunofluorescence staining results in about 3-fold signal amplification compared to direct immunofluorescence staining.

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Labeled secondary antibodies will still bind to primary antibodies labeled using Super-n-Stain™ kits, therefore a secondary antibody cannot be used to distinguish an unlabeled primary antibody from a Super-n-Stain™ labeled primary antibody from the same species. Super-n-Stain™ labeled antibodies can be used as a tertiary staining antibody after standard immunofluorescence staining with primary and secondary antibodies. We also provides Super-n-Stain™ labeling kit with biotin, rifavidin labeled with YF® Dye for secondary detection or monoclonal biotin antibody labeled with YF® Dye.

Protocol

Preparation

Super-n-Stain™ Antibody Labeling Kits is suitable for IgG labeling. We do not recommend labeling other proteins because the labeled DOL(degree of labeling) may not reach a good value. Changes of Super-n-Stain™ Labeling conditions may cause IgM antibody denaturation.

If you don't know the concentration or composition of antibody, contact the antibody supplier to get the answers before trying to label your antibody. Super-n-Stain™ can not be used to label natural antiserum or culture supernatant of hybridoma cell line. Purify IgG with protein purification steps or commercial antibody purification kits, such as Pierce Antibody Clean-Up Kit.

The antibody solution without stabilizers can get better labeling results, while the standard labeling procedure can be compatible with low concentration of BSA, gelatin, Tris and glycerin, and the labeling solution won't be affected by NaN3. For the standard labeling protocol (B), select the specification of the kit according to the μg quantity of IgG to be labeled.

The modified labeling protocol (C) of Super-n-Stain™ is based on the total amount of labeled protein rather than the amount of IgG. Modified labeling protocol applied to antibody labeling with too many stable proteins, antibody labeling in solution is also suitable for modified labeling protocol. However, you must determine the concentration of total protein in the solution before labeling.(protein concentration is estimated by measuring absorbance in 280 nm). Selection of kit specifications is based on total protein quantity of your labeled antibody sample. The modified labeling protocol can also be used to label samples whose IgG content is lower than the lowest range of the kit,total protein quantity is within the labeling range of the kit by adding stabilizer protein.

The antibody with low concentration of BSA or gelatin may have a slightly stronger staining background than the antibody without these stabilizers. If antibody labeling is carried out in the presence of BSA or gelatin, the use of blocking and cleaning agents containing 1% BSA or gelatin will greatly reduce the staining background.

You can use the ultrafiltration tube provided in the kit to purify your antibody to remove nonprotein component such as Tris, glycine or glycerin, you can use the ultrafiltration tube provided in the kit to purify your antibody, as follows step(A).

Concentration of antibody with high labeling efficiency was 0.5-1 mg/mL.Ultrafiltration tube can be used to concentrate antibody(Note: stable protein will also be concentrated in ultrafiltration bottle).We provide WonderOrange™ Protein Quantitation Kit(W6006) to quantify antibodies of unknown concentrations.If the antibody does not need to be removed or concentrated, experiments according to standard labeling protocol (B) or modified labeling protocol (C).

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Table 1. Antibody compatibility and labeling step selection guide of Super-n-Stain™

Component	Compatibility
NaN_3	Compatible
Glycerol	≤10%：Standard labeling protocol (Part of step B) ＞10%：Execute ultrafiltration protocol (Part of step A)
Tirs	≤20 mM：Standard labeling protocol (Part of step B) ＞20 mM：Execute ultrafiltration protocol (Part of step A)
Glycine	Execute ultrafiltration Protocol (Part of step A)
BSA or gelatin	≤less than 4:1 BSA or gelatin to IgG by µg amount：Standard labeling protocol（(Part of step B) ＞more than 4:1 BSA or gelatin to IgG by µg amount：Modified labeling protocol (Part of step C)
Ascites	Modified labeling protocol (Part of step C)
Serum	Incompatible：Purified IgG
Culture supernatant of hybridoma cell line	Incompatible：Purified IgG

A.Ultrafiltration Protocol

Important: Before you begin, complete the guide(Table 1) to determine whether your antibody is compatible with labeling, and to choose the right labeling protocol. If necessary, contact the antibody manufacturer for concentrations of IgG and antibody stabilizer. The molecular weight retained by the ultrafiltration membrane is 100000, so that small than 10 kDa will pass through the membrane, and molecules larger than 10 kDa, including IgG, will remain on the membrane (Fig. 1). Be careful not to touch the membrane with straw, which can tear or puncture the membrane, resulting in the loss of antibodies.

Ultrafiltration Vial Capacities

Maximum Sample Volume: 500 µL (see note above)

Final Concentrate Volume: 15 µL

Filtrate Receiver Volume: 500 µL

Hold-up Volume (Membrane/Support): < 5 µL

1 Add an appropriate amount of antibody to the membrane of the ultrafiltration vial, being careful not to touch the membrane. Centrifuge the solution at 14,000 xg 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. Remove the flow-through liquid from the collection tube.

Note: We recommend saving the filtrate solutions after steps 1 and 2, so you can recover your antibody in case of membrane failure during centrifugation.

2 To concentrate your antibody, proceed to step 3. To remove interfering substances, add an equal volume of 1× PBS to the membrane. Spin the vial at 14,000 xg until the liquid has filtered into the collecting tube.

3 Add an appropriate concentration of PBS to the membrane to obtain a final antibody concentration of 0.5-1 mg/mL. Carefully pipet the PBS up and down over the upper surface of the membrane to recover and resuspend the antibody.

4 Transfer the recovered antibody solution to a fresh microcentrifuge tube.

5 If you are using Protocol C (Modified Labeling Protocol),

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save the ultrafiltration vial to concentrate your antibody after labeling. Additional ultrafiltration vials also can be purchased separately.

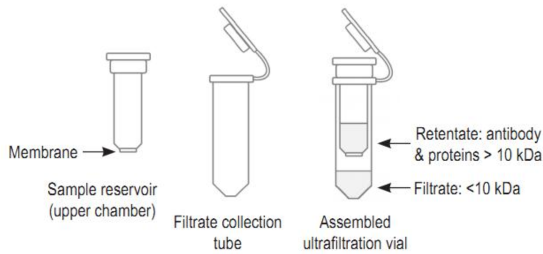

Fig.1 Components of ultrafiltration vial

B. Standard Super-n-Stain™ Labeling Protocol

Important: Before you begin, complete the guide(Table 1) to determine whether your antibody is compatible with labeling, and to choose the right labeling protocol.

1. Start with your antibody at 0.5-1 mg/mL IgG in a compatible buffer. Transfer an appropriate amount of antibody to be labeled to a clean tube.

2. Warm up the Super-n-Stain™ reaction buffer and the Super-n-Stain™ storage buffer to room temperature before use. Centrifuge briefly to collect the solutions at the bottom of the vials.

3. Mix the 10× Super-n-Stain™ Reaction Buffer with the antibody solution at a ratio of 1:10 so that the antibody solution contains a final concentration of 1× Reaction Buffer (for every 9 μL of antibody solution, add 1 μL of 10× reaction buffer). Mix completely by pipetting up and down or gentle vortexing.

4. Transfer the entire solution from Step 3 to the vial containing the dye/label (Component A). There is no need to measure the amount of the dye/label in the vial. Vortex the vial for a few seconds.

5. Incubate for 30 minutes at room temperature and protect from light.

6. Dilute the labeled antibody solution with the provided storage buffer. Simply transfer the entire labeled antibody solution into the storage buffer vial and mix. The antibody is now ready to use for staining. The concentration of the labeled antibody is the amount of your starting antibody divided by the total volume. Antibody storage buffer contains 2 mM sodium azide.

7. The labeled antibody can be stored stably for at least 6 months at 4 ℃ and protect from light. In addition, it can be stored in multiple small packages at -20 ℃ for a long time. Note: if you prefer not to use the antibody dilution buffer, you can pack the solution into several small packages and store it at -20 ℃ to avoid repeated freezing and thawing. The labeled antibody can be stored stably for at least 6 months.

C. Modified Super-n-Stain™ Labeling Protocol

Important: Before you begin, complete the guide(Table 1) to determine whether your antibody is compatible with labeling, and to choose the right labeling protocol.

1. Start with your antibody at 0.5-1 mg/mL total protein in a compatible buffer, it should be dissolved or diluted with PBS if necessary. Make sure the total μg of the protein matches the labeling range of the kit. If the IgG to be labeled is less than the minimum labeling amount of the kit, add BSA to make the total protein of BSA and IgG match the kit.

2. Warm up the Super-n-Stain™ reaction buffer and the Super-n-Stain™ storage buffer to room temperature before use. Centrifuge briefly to collect the solutions at the bottom of the vials.

3. Mix the 10× Super-n-Stain™ Reaction Buffer with the antibody solution at a ratio of 1:10 so that the antibody solution contains a final concentration of 1× Reaction Buffer (for every 9 μL of antibody solution, add 1 μL of 10× reaction buffer). Mix completely by pipetting up and down or gentle vortexing.

4. Transfer the entire solution from Step 3 to the vial containing the dye/label (Component A). There is no need to measure the amount of the dye/label in the vial. Vortex the vial for a few seconds.

5. Incubate for 30 minutes at room temperature and protect

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from light.

6. Optional: you can transfer the entire labeling reaction to the tube of antibody storage buffer provided. However, this may result in a highly dilute IgG solution, which may not be practical for subsequent use. Transfer the antibody to the storage buffer without additional dilution and execute as follow steps below. Antibody storage buffer contains 2 mM sodium azide.

7. Transfer the labeling reaction to the membrane of the ultrafiltration vial provided (or saved after antibody clean-up, above). Centrifuge the vial at 14,000 xg until all of the liquid has filtered into the receiving vial as described in Protocol A.

8. Add an appropriate volume of antibody storage solution to the ultrafiltration tube membrane, and carefully pump PBS up and down on the membrane surface to suspend the antibody again. Antibody storage buffer contains 2 mM sodium azide.

9. Transfer the recovered antibody solution to a fresh microcentrifuge tube. The antibody is now ready to use for staining.

10. The labeled antibody can be stored stably for at least 6 months at 4 ℃ and protect from light. In addition, it can be stored in multiple small packages at -20 ℃ for a long time.

Frequently Asked Questions of Super-n-Stain™ Antibody Labeling Kits

Question	Answer
Do I need to remove unconjugated free dye from the labeled antibody since there is no purification step?	Because of the unique formulations of our dyes and labeling technology, it is not necessary to remove unconjugated free dye before staining. However, ultrafiltration can be used to remove free dye if desired. We recommend performing ultrafiltration before adding antibody storage buffer.
Can I use Super-n-Stain™ labeled antibodies for multi-color immunofluorescence staining, or will the dye transfer between antibodies?	Super-n-Stain™ labeling results in covalent linkage of dye and antibody, so there will be no dye diffusion or transfer between labeled antibodies or the target sample.
Is staining with Super-n-Stain™ labeled antibodies as sensitive as staining with unlabeled primary and fluorescent secondary antibodies?	Direct immunofluorescence detection can be less sensitive than indirect detection.
What are the advantages of using directly labeled conjugates compared to indirect staining with labeled secondary antibodies?	Direct immunofluorescence staining eliminates the need for secondary antibody incubation, and allows the use of multiple primary antibodies from the same species for multicolor detection, or staining of animal tissues with antibodies raised in the same species (e.g. mouse-on-mouse).
Can I split the kit contents and use it more than one time?	No. The Super-n-Stain™ kits are optimized for 1 labeling. We do not recommend that you try to split the kit to label more than one antibody or for more than one use.

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