StreptaClick®-color antibody labeling kit

Size: 500 µl (labels 50 µg biotinylated antibody)

Storage: +4 °C, protect from light

Kit content

- StreptaClick® antibody labeling reagent, 500 µl, contains 0.09% sodium azide
- Biotin block buffer, 500 µl (biotin in PBS)

This document contains protocols for (A) antibody labeling with StreptaClick®-color, and (B) the use of StreptaClick®-labeled antibodies for immunostaining of samples.

Introduction

StreptaClick®-color provides a rapid, robust, and flexible method for attaching fluorochromes to biotinylated antibodies. The antibody-StreptaClick® complex (StreptaClick®-labeled antibody) is stable and can be used in the same way as antibodies conjugated directly with fluorochromes.

StreptaClick®-color is a unique immunolabeling tool as it enables multiplexing with multiple biotinylated antibodies. There is no spillover of StreptaClick® reagent between antibodies and antibodies of the same animal host species can be used.

The StreptaClick® antibody labeling reaction is efficient and can be run in such small volumes that it becomes practical to label individual antibodies with fluorochromes depending on the experiment.

Storage and Handling

Upon receipt, store the StreptaClick®-color antibody labeling kit at +4 °C. The kit can be stored for 6 months after receipt. Do not freeze. Protect fluorescent components from light. **Note:** The StreptaClick® antibody labeling reagent contains 0.09% sodium azide.

Applications

StreptaClick®-labeled antibodies can be used for immunostainings in histology and flow cytometry. Other immunostaining applications have not been tested. StreptaClick®-labeled antibodies can be combined with standard immunolabeling protocols, such as primary + secondary antibodies or directly conjugated antibodies, but not with conventional streptavidin.

Before you begin

If the antibody is biotinylated 'in house' using a biotinylation kit, excess free biotin must be removed (e.g. by a spin column). Occasionally, antibodies have lysine residues that are used for biotinylation near the antigen-binding site. In these cases, StreptaClick® can sterically affect the binding to the antigen. Kits that specifically biotinylate the Fc-portion of the antibody will circumvent this potential problem.

The StreptaClick® antibody labeling step (A) can be performed several days in advance of immunostaining step (B). **Note:** When Biotin block buffer is added, the StreptaClick®-labeled antibody should be used within 8 hours.

The StreptaClick® antibody labeling step (A) can be performed at room temperature (RT) or at +4 °C.

The antibody-to-StreptaClick® ratio is important. Check the concentration of the antibody. Avoid volumes <2 µl to achieve correct amounts of the components. Pre-dilute the antibody and/or the StreptaClick® antibody labeling reagent with PBS when volumes become small. Dilution does not influence labeling efficiency.

The StreptaClick® antibody labeling reaction does not require removal of bovine serum albumin (BSA) or other stabilizing proteins that may be present in antibody preparations.

Do not combine StreptaClick®-color with protocols where conventional streptavidin is used.

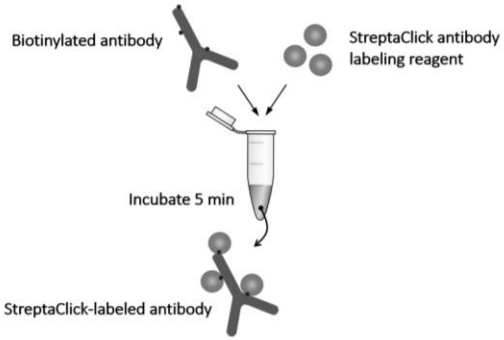
The StreptaClick®-color fluorochromes have the following excitation (Ex) and emission (Em) peaks:

- StreptaClick®-color, AZDye™488: Ex 495 nm, Em 520 nm
- StreptaClick®-color, Atto542*: Ex 542 nm, Em 562 nm
- StreptaClick®-color, AZDye™ 594: Ex 590 nm, Em 620 nm
- StreptaClick®-color, AZDye™ 647: Ex 650 nm, Em 668 nm

** Atto542 is a Cy3 equivalent*

Caution: Make sure what fluorochromes that can be simultaneously separated by your microscope. 594 fluorochromes may overlap with both 542 and 647 fluorochromes. All four fluorochromes can simultaneously be used without spectral overlap using the SpectraSplit® filter sets from Kromnigon AB (kromnigon.com).

(A) StreptaClick® antibody labeling protocol



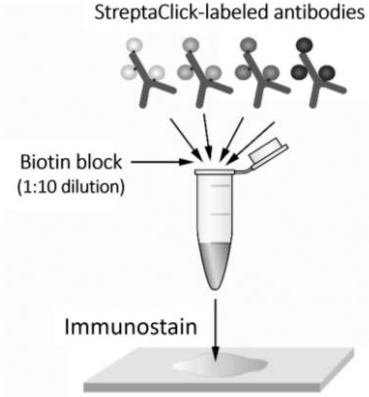
Steps:

1. **Add the desired amount of biotinylated antibody to be labeled to a suitably sized tube.** Pre-dilute the antibody if the volume is less than 2 ul. Make sure that all the antibody solution is at the bottom of the tube. Antibody concentration as low as 0.01 mg/ml can be used.
2. **Add the appropriate amount of StreptaClick® antibody labeling reagent (see table below) and mix immediately by pipetting up and down**. **Avoid bubbles**. The volume of StreptaClick® antibody labeling reagent should preferably be larger than the volume of the antibody. Pre-dilute the StreptaClick® antibody labeling reagent if the volume is small.

antibody concentration	µl antibody	µl StreptaClick antibody labeling reagent
1 mg/ml	1	10
0.5 mg/ml	1	5
0.1 mg/ml	1	2
0.1 mg/ml	1	1

3. **Incubate for 5 minutes at +4 or +20 °C**. The StreptaClick®-labeled antibodies are now ready to be used for immunostaining procedures or can be stored for at least one week at +4°C until use, provided that the antibody *per se* tolerates the storage.

(B) StreptaClick® immunostaining protocol



The StreptaClick®-labeled antibodies can be used for multi-immunostaining similar to directly conjugated antibodies. When multiplexing with several StreptaClick®-labeled antibodies, the immunostaining solution must contain Biotin block to inactivate any free StreptaClick® antibody labeling reagent.

Steps:

Prepare your tissue samples for immunostaining according to standard protocols. There are no restrictions for pre-blocking reagents (e.g. serum, IgGs, BSA, Fc-block). There is no need for avidin/biotin blocking step.

1. **Add 10% Biotin block buffer to the immunostaining solution for multistainings.** The Biotin block buffer will efficiently quench any excess reactive StreptaClick® antibody labeling reagent. The amount of Biotin block can vary between 5-15%.
2. **Add StreptaClick®-labeled antibodies (and other antibodies) one by one into the immunostaining solution.** As a guideline: use the same amount of antibody or up to twice as much as in standard immunostaining protocols. StreptaClick®-labeled antibodies can be combined with other primary antibodies.
3. **Apply the immunostaining solution to your samples and incubate.** Continue the immunostaining procedure according to standard protocols. As a guidance incubate 0.5-2 hours at RT or overnight at +4°C.
4. **Wash, mount, and analyze under a fluorescence microscope.** Make sure what fluorochromes that can be separated by your fluorescence microscope without spectral overlap (see 'Before you begin' on page 1).

Trouble shooting guide

The brightness of antibodies labeled with StreptaClick®-color is similar to directly conjugated antibodies.

Weak/No signals

- Check that your biotinylated antibody works using a 2-step immunostaining with StreptaClick®-color. Incubate your sample with the biotinylated antibody, wash, and then add StreptaClick® antibody labeling reagent (diluted 1:40 in PBS) in a separate second step.

- Some antibodies have biotin conjugated near the antigen-binding site, resulting in sterical hindrance when attaching StreptaClick® to the antibody. Reducing the amount of added StreptaClick® antibody labeling reagent by 30-50% in the antibody labeling step (A) will then improve the signal.

- Take care that the entire StreptaClick® antibody labeling reagent during the labeling step is rapidly mixed with the biotinylated antibody without formation of bubbles. It is easier to mix rapidly if the StreptaClick® reagent volume is >2 times the antibody volume.

- The antibody-to-StreptaClick® reagent ratio is important. Check that the concentration of the biotinyated antibody is correct.

- Increase the amount of StreptaClick®-labeled antibody used during the immunostaining step (B).

- Consider changing fluorochrome. AZDye™ 647 has the highest signal-to-noise ratio.

High background signals

- Use less StreptaClick® antibody labeling reagent during the labeling step (A).

- Lower the amount of antibody during the immunostaining step (B).

- The primary antibody may have aggregates. Spin the biotinylated antibody at full speed in a tabletop centrifuge (~21000 x g) for 10 minutes before labeling with StreptaClick®, to remove such antibody aggregates.

- Wash with 0.01% Triton-X100 after the antibody incubation step of the sample.

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