StreptaClick-color antibody labeling kit

Size: 500 µl (labels 50 µg biotinylated antibody)

Storage: +4 ºC, protect from light

Kit content

- StreptaClick 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, and (B) the use of StreptaClick-labeled antibodies for immunostaining of samples.

Introduction

The StreptaClick-color antibody labeling kit provides a rapid and flexible method for attaching fluorochromes to biotinylated antibodies. The formed complex between the antibody and StreptaClick is stable and can be used in the same way as antibodies that are directly conjugated with fluorochromes.

The StreptaClick labeling reaction is efficient and can be run in such small volumes that it becomes practical to label individual antibodies with fluorochromes prior to each experiment. The labeled antibodies can then med mixed and used for multiplex immunohistochemistry (IHC)

There is no spillover of StreptaClick reagent between antibodies and antibodies of the same animal host species can be used.

Storage and Handling

Upon receipt, store the StreptaClick antibody labeling kit at +4 ºC. The kit can be used for at least 6 months after receipt. Do not freeze. Protect fluorescent components from light. Note: The StreptaClick 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 streptavidin.

Before you begin

If the antibody is biotinylated 'in house' using a biotinylation kit, excess free biotin must be removed (eg by a spin column). Some antibodies have biotin conjugated 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 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 must be used within 24 hours.

The StreptaClick labeling step (A) can be performed at room temperature (RT) or at +4 ºC.

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 reagent with PBS when volumes become too small. Dilution does not influence labeling efficiency.

The StreptaClick 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-labeled antibodies with protocols where standard streptavidin is used.

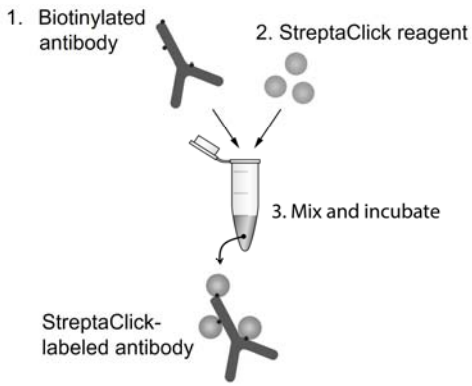
The StreptaClick fluorochromes have the following excitation (Ex) and emission (Em) peaks:

- StreptaClick, AlexaFluor488: Ex 495 nm, Em 520 nm
- StreptaClick, Atto542*: Ex 542 nm, Em 562 nm
- StreptaClick, AlexaFluor 594: Ex 590 nm, Em 620 nm
- StreptaClick, AlexaFluor 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



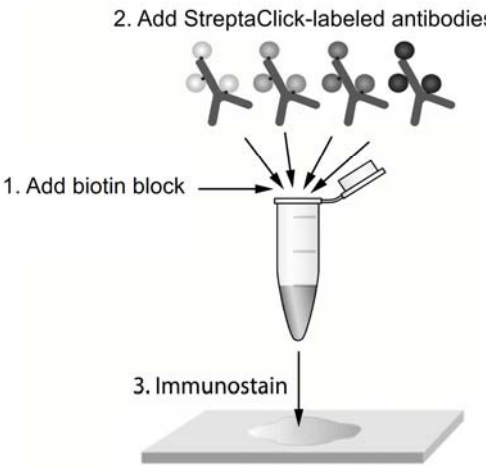
Steps:

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 of the antibody solution is at the bottom of the tube. Antibody concentration as low as 0.01 mg/ml can be used. 0.2 ml PCR tubes are convenient to use for small reactions.
2. Add the appropriate amount of StreptaClick reagent (see table) and mix immediately by pipetting up and down. Avoid bubbles. Adding StreptaClick reagent above the surface of the antibody solution is the most efficient way to obtain a rapid even mixture. Pre-dilute the StreptaClick reagent up to 10 times if using small volumes (>2 ul). The volume of StreptaClick should preferably be larger than the volume of the antibody.

Antibody concentration	µL antibody	µL FlexiStain reagent	Dilution
1 mg/mL	1	10	11 times
0.5 mg/mL	1	5	6 times
0.2 mg/mL	1	2	3 times
0.1 mg/mL	1	1	2 times

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

(B) Immunostaining protocol



The StreptaClick-labeled antibodies can be used for multiplex IHC similar to directly conjugated antibodies. When multiplexing with several StreptaClick-labeled antibodies, the immunostaining solution shall contain biotin block buffer to inactivate any free StreptaClick reagent. The biotin block buffer is not necessary if only one StreptaClick-labeled antibody is used.

Steps:

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

Add 10% Biotin block buffer to the immunostaining solution for multistainings. The Biotin block buffer quenches any excess reactive StreptaClick. The amount of Biotin block can vary between 5-10%. If convenient, Biotin block buffer can be added to each StreptaClick-labeled antibody after the labeling reaction (step 3 in protocol A). Use approximately 0.5 µl biotin block / µg antibody. When biotin has been added, immunolabeling should be performed within 8 hour.

1. 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. The table on page 1 shows how much the antibody has been diluted during the StreptaClick labeling step. StreptaClick-labeled antibodies can be combined with other primary antibodies. Continue to the next step within 24 hours when Biotin block has been added.
2. Apply the immunostaining solution to your samples and incubate. Continue the immunostaining procedure according to standard protocols. For immunostaining of tissue sections, the incubation times normally are 0.5-3 hours at RT or overnight at +4°C.
3. 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. Incubate your sample with the biotinylated antibody, wash, and then add StreptaClick reagent (diluted 1:40 in PBS) in a separate second step.

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

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

- Take care that the entire StreptaClick reagent is rapidly mixed with the biotinylated antibody without formation of bubbles during the labeling step. Adding StreptaClick on top of the antibody solution facilitates a rapid mixture. It is easier to mix rapidly if the StreptaClick 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.

- Consider changing fluorochrome. StreptaClick-color 542 is the brightest color. StreptaClick-color 647 has the highest signal to noise ratio.

High background signals

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

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

- Spin the biotinylated antibody at full speed on a table top centrifuge (~21000 x g) for 10 minutes before labeling with StreptaClick, to remove potential antibody aggregates.

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