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ab272188 Streptavidin Atto 542 (Monovalent)

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www.abcam.com/ab272188

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For the attaching fluorochromes to biotinylated antibodies.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Streptavidin Atto 542 (Monovalent)(ab272188) provides a rapid, robust, and flexible method for attaching fluorochromes to biotinylated antibodies. The antibody- Streptavidin Atto 542 (Monovalent) complex is stable and can be used in the same way as antibodies conjugated directly with fluorochromes.

Streptavidin Atto 542 (Monovalent) is a unique immunolabeling tool as it enables multiplexing with multiple biotinylated antibodies. There is no spillover of Streptavidin Atto 542 (Monovalent) reagent between antibodies and antibodies of the same animal host species can be used.

The Streptavidin Atto 542 (Monovalent) 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.

Kits are available with 4 different fluorescent labels:

Streptavidin Alexa Fluor® 488 Equivalent (Monovalent) (ab272187)

Streptavidin Atto 542 (Monovalent) (ab272188) - this kit

Streptavidin Alexa Fluor® 594 (Monovalent) (ab272189)

Streptavidin Alexa Fluor® 647 Equivalent(Monovalent) (ab272190)

2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. Do not freeze.

Item	Quantity		Storage temperature
	25 µg kit	50 µg kit	
Streptavidin Atto 542 labeling reagent (Monovalent)	250 µL	500 µL	+4°C
Biotin block buffer	250 µL	500 µL	+4°C

Δ Note: Protect reagents from light.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Biotinylated antibody of interest
- Spin columns (if free biotin needs to be removed from antibody prior to labeling)
- Microfuge or PCR tubes
- PBS
- Fluorescent microscope (with appropriate filters; see Section 5)
- Tissue samples

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Before You Begin - Antibody Considerations

5.1 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 Streptavidin Atto 542 can sterically affect the binding to the antigen. Kits that specifically biotinylate the Fc-portion of the antibody will circumvent this potential problem.

5.2 The labeling step (Section 6) can be performed several days in advance of immunostaining step (Section 7).
Δ Note: Once Biotin block buffer is added, the labelled antibody must be used within 24 hours.

5.3 The labeling step (Section 6) can be performed at room temperature (RT) or at +4 °C.

5.4 The antibody-to-labeling reagent 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 labeling reagent with PBS when volumes become small. Dilution does not influence labeling efficiency.

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

5.6 Do not combine Streptavidin Atto 542 with protocols where standard streptavidin is used.

5.7 Excitation and emission peaks for the four labels that are available in different kits:

- (ab272187) AZDye 488: Ex 495 nm, Em 520 nm
- (ab272188) Atto542: Ex 542 nm, Em 562 nm
- (ab272189) Alexa Fluor® 594: Ex 590 nm, Em 620 nm
- (ab272190) AZDye 647: Ex 650 nm, Em 668 nm

Δ Note: Atto542 is a Cy3 equivalent. AZDye 488 is an AlexaFluor® 488 equivalent. AZDye 647 is an AlexaFluor® 647 equivalent.

5.8 Determine which fluorochromes 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 appropriate filter sets.

6. Labelling protocol

6.1 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 µL. 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.

6.2 Add the appropriate amount of labeling reagent (see table, below) and mix immediately by pipetting up and down. Avoid bubbles. Adding labeling reagent above the surface of the antibody solution is the most efficient way to obtain a rapid even mixture. Pre-dilute the labeling reagent up to 10 times if the volume is small. The volume of labeling reagent should preferably be larger than the volume of the antibody.

Antibody concentration	Antibody (µL)	Labeling Reagent (µL)	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

6.3 Incubate for 5 minutes at RT or at +4°C. The labeled antibody is 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.

7. Staining Protocol

The labeled antibodies can be used for multi-immunostaining similar to directly conjugated antibodies. When multiplexing with several antibodies labeled using one these kits, the immunostaining solution must contain Biotin block to inactivate any free labeling reagent. The Biotin block buffer is not necessary if only one labeled antibody is used.

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). Antibodies labeled using this kit do not need an avidin/biotin blocking step.

7.1 Add 10% Biotin block buffer to the immunostaining solution for multi-stainings. The Biotin block buffer quenches any excess reactive labeling reagent. The amount of Biotin block can vary between 5-10%. If convenient, Biotin block buffer can be added to each labeled antibody after step 6.3 (approximately 0.5 µL biotin block / µg antibody; must be used within 24 hours, see step 7.2).

7.2 Add antibodies labeled using this kit protocol (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 in step 6.2 shows how much the antibody has been diluted during the labeling step. Labeled antibodies can be combined with other primary antibodies. Continue to the next step within 24 hours when Biotin block has been added.

7.3 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.

7.4 Wash, mount, and analyze under a fluorescence microscope. Make sure which fluorochromes can be separated by your fluorescence microscope without spectral overlap (see step 5.8).

Δ Note: The brightness of antibodies labeled with this kit is similar to directly conjugated antibodies.

Δ Note: In the case of weak / no signals consider the following:

- Some antibodies have biotin conjugated near the antigen-binding site, resulting in sterical hindrance when attaching the label to the antibody. Reducing the amount of added labeling reagent by 50% in the labeling step (Section 6) will then improve the signal.
- Increase the amount of labeled antibody used during the immunostaining step (Section 7).
- Some antibodies have biotin conjugated near the antigen-binding site, resulting in sterical hindrance when attaching the label to the antibody. Reducing the amount of added labeling reagent by 50% in the labeling step (Section 6) will then improve the signal.
- If the signal is weak, increase the amount of labeled antibody used during the immunostaining step (Section 7).
- Take care that the entire labeling reagent during the labeling step is rapidly mixed with the biotinylated antibody without formation of bubbles. Adding the labeling reagent on top of the antibody solution facilitates a rapid mixture. It is easier to mix rapidly if the labeling reagent volume is >2 times the antibody volume.
- Consider changing fluorochrome. AlexaFluor® 647 has the highest signal to noise ratio.

Δ Note: In the case of high background signal, consider the following:

- Use less labeling reagent during the labeling step (Section 6)
- Lower the amount of antibody during the immunostaining step (Section 7)
- Spin the biotinylated antibody at full speed on a table top centrifuge (~21,000 x *g*) for 10 minutes before labeling, to remove potential antibody aggregates.
- Wash with 0.01% Triton X100 after the antibody incubation step of the sample

8. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)