ab312827 – Multiplex IHC Detection kit (TSA amplification)

For research use only - not intended for diagnostic use. This kit provides 90 (small) to 250 (large) stainings.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab312827

Overview

The StreptaClick–HRP Multiplex IHC kit provides a powerful method for multiplex immunohistochemistry using tyramide signal amplification (TSA) on frozen tissue sections. The method is based on horseradish peroxidase (HRP) that with a click reaction is attached to biotinylated antibodies using a processed form of streptavidin conjugated with HRP (StreptaClick–HRP). The HRP–labeled biotinylated antibodies can then be used for multiple cycles of immunostainings where each fluorochrome is sequentially developed by TSA (TSA reagents are not provided in the kit). The kit contains a HRP block buffer optimized for preserving the morphology of frozen tissue sections. The HRP block buffer rapidly quenches the HRP enzyme at room temperature after each TSA cycle, and allows sequential multiplex IHC of up to six antibodies in one day.

Storage and Stability

The kit components are stored at 4ºC upon arrival.

Materials Supplied

Item	Quantity (90 stainings)	Quantity (250 stainings)	Storage Condition
StreptaClick-HRP	170 µl	500 µl	4ºC
Biotin block buffer	300 µl	600 µl	4ºC
HRP block buffer	9 ml	25 ml	4ºC
3% H202	100 µl	300 µl	4ºC
Block activator	1.5 ml	3 ml	4ºC

Materials Required, Not Supplied

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

- Reagents for TSA (tyramide fluorochromes and tyramide amplification buffer).
- Biotinylated antibodies.
- Spin columns (if free biotin needs to be removed from antibody prior to labelling).
- Microfuge tubes.
- PBS.
- Fluorescent microscope (with appropriate filters).
- Tissue samples.

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.

Assay Procedure

- If the antibody is biotinylated 'in house' using a biotinylation kit, excess free biotin must be removed before use (e.g. by a spin column).
- Do not use dry milk in the immunostaining buffer. It may contain free biotin that can quench StreptaClick–HRP during the HRP labeling step. If desired, dry milk can be added after the HRP labeling reaction.
- The HRP labeling reaction is not affected by BSA or other stabilizing proteins that may be present in antibody preparations.
- The ratio between biotinylated antibody and StreptaClick–HRP is important for optimal HRP labeling (see Table below). Make sure that you know the approximate antibody stock concentration. To avoid pipetting errors, use intermediate dilution if using less than 1 µl of the antibody stock solution.

Antibody stock concentration (µl)	Antibody (µl)	StreptaClick-HRP (µl)	Biotin block buffer (µl)
1 mg/mL	1	20	20
0.5 mg/mL	1	10	10
0.1 mg/mL	1	2	2

Note: The HRP labeling reaction is performed at room temperature (RT). Multiple biotinylated antibodies can be labeled with HRP in parallel. Store HRP-labeled antibodies at +4ºC, and use them for TSA immunostaining within 8 hours.

HRP Labeling protocol

- The HRP labeling protocol attaches HRP to biotinylated antibodies that will be used for TSA immunostaining.

1. Place two tubes of the same size in a rack (e.g. 1.5 ml microfuge tubes).
2. In the first tube, add the desired amount of biotinylated antibody to be used for TSA immunostaining. Avoid pipetting volumes of <1 µl.
3. Dilute the antibody with immunostaining buffer of choice to the working concentration that will be used in the TSA immunostaining. As a guidance, use a 1:500-1:1500 dilution from a 1 mg/ml antibody stock solution. Pre-diluting the antibody to a working concentration before mixing with StreptaClick-HRP avoids HRP quenching by sodium azide that is often used as a preservative in antibody stock solutions.
4. To the second tube, add the appropriate amount of StreptaClick–HRP according to the ratio table in the previous section.
5. Transfer all antibody solution from the first tube to the second tube and mix immediately by pipetting up and down. Avoid bubbles.
6. After 10 minutes or more, add Biotin block buffer according to Table and mix. The Biotin block buffer immediately inactivates any remains of active StreptClick–HRP. The biotinylated antibody is now labeled with HRP and ready to be used for TSA immunostaining.

TSA immunostaining protocol

The HRP-labeled antibodies can be used for multiplex TSA immunostaining. The protocol does not use heat treatment between cycles, which allows TSA multiplex immunostaining on frozen tissue sections. Each staining cycle contains three main procedures – Antibody incubation, Color development, and HRP block.

1. Prepare your tissue sections for immunostaining according to standard protocols. There is no need for avidin/biotin blocking.
2. Block endogenous peroxidases with the provided HRP block buffer. Add 1 µl 3% H2O2 and 100 µl Block activator to each 100 µl of HRP block buffer and apply to the tissue

sections. Incubate for 10 minutes at RT. The activated HRP block buffer will also be used in step 5 and can be stored at +4ºC in the dark for 8 hours.
3. Apply HRP-labeled antibody to your tissue sections and incubate 20-45 minutes at RT. Wash x3 in PBS.
4. Dilute tyramide fluorochrome in tyramide amplification buffer and apply to your samples. Tyramide reagents are not provided in the kit. Incubate 10 minutes at RT and wash x2 in water or PBS.
5. Apply activated HRP block buffer to the tissue sections and incubate for 10 minutes at RT. This step is only needed when the immunostaining procedure is continuing with a new antibody cycle. Wash x3 in PBS.
6. Repeat steps 3 and 5 with the next HRP-labeled antibody.
7. Wash, mount, and analyze under a fluorescence microscope.

Note: In the case of weak signals consider the following:
- Check that your biotinylated antibody works using a two-step immunostaining with the StreptaClick–HRP; 1) Incubate tissue section with the biotinylated antibody. 2) Wash and incubate tissue sections with StreptaClick–HRP alone, diluted 1:20 in PBS. Wash and develop color with TSA.
- The ratio between biotinylated antibody and StreptaClick–HRP is important. Check the concentration of the antibody. If you do not know the exact concentration, test different amounts of labeling reagent during antibody labeling.
- It is important to first prepare an antibody working solution before mixing with StreptaClick–HRP. Pre-diluting to a working concentration avoids HRP quenching by sodium azide that is often used as a preservative in antibody stock solutions.
- Increase the amount of antibody used during the immunostaining step.
- Increase the amount of tyramide fluorochrome during the color developing step. Twice the amount of tyramide fluorochrome can improve the signal significantly.
- Carefully wash after the HRP block step (TSA immunostaining protocol, step 5). The HRP block buffer may not have been washed away properly before adding the next HRP–labeled antibody to the sample.
- Some antibodies have biotin conjugated near the antigen-binding site, resulting in sterical hindrance when attaching the HRP to the antibody. Using less StreptaClick–HRP during the labeling step may help.
- Do not use dry milk in the immunostaining buffer, since it may contain free biotin that quenches the StreptaClick–HRP. If desired, dry milk can be added after the antibody labeling reaction.

Note: In the case of cross-over signal between antibodies consider the following:
- The HRP block step may be incomplete (TSA immunostaining protocol, step 5). Prolong the incubation time to 15 min. Check that H2O2 and Block activator are properly added to the HRP block buffer.
- The biotin block step may be incomplete (HRP Labeling protocol, step 6). Check that the Biotin block buffer is properly added before applying HRP-labeled antibodies to the tissue.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus