Rabbit/Mouse Universal Streptavidin-HRP Kit (DAB)

Project number: S665512

Storage conditions: 2-8° C.

Product Content:

Component	S665512 3preps
BlockingBuffer	300ml
AntibodyPretreatSolution (HRP/Mouse)	3ml
DilutionBuffer	300ml
WashBuffer (10×)	00ml

Product Description:

This kit is designed according to the principle that Biotin has strong affinity with Streptavidin. After the primary antibody of rabbit or mouse origin binds to the corresponding target antigen, the biotinylated antibody in this kit--rabbit/mouse universal secondary antibody binds specifically to the primary antibody; the biotin labeled on the secondary antibody binds to the streptavidin labeled with HRP, forming an antigen-specific primary antibody-biotinylated secondary antibody-HRP-labeled streptavidin complex. The HRP catalyzes the color development of the substrate, thus inferring the presence and distribution of the antigen to be examined. The biotinylated secondary antibody and SA-HRP used in this kit are optimized for labeling and purification, resulting in higher sensitivity and lower background for staining, and are suitable for the detection of formalin-fixed, paraffin-embedded tissue sections, as well as frozen sections, cell crawls, and freshly prepared blood smears. The Rabbit/Mouse Universal Streptavidin-HRP Kit is suitable for use with CombiSense ready-to-use or concentrated antibodies.

Matters needing attention:

1. Calculated by adding 1 drop (about 50 μl) per slide, 3 ml can do 60 slices, 18 ml can do 360 slices.
2. For tissues that are rich in endogenous biotin, it is preferable to use this kit with an endogenous biotin blocker for containment.
3. DAB working solution is ready to use, the prepared working solution is effective within 1 hour at 2-8° C and protected from light.
4. Avoid drying out of the tissue slices during the experiment, so the amount of working solution used in each step of the incubation must be sufficient to ensure complete coverage of the tissue samples and the incubation should be carried out in a wet box as much as possible.
5. In order to obtain the best results, please make sure to optimize the experimental conditions and the amount of reagents.
6. DAB is a suspected carcinogen, please take necessary protective measures when using it.

7. This product is intended for scientific research only and is not intended for human reaction or human treatment.

Operation steps:

1. Routinely process samples such as paraffin or frozen tissue sections or cell crawls for testing.

(1) Tissue sections or cell crawler sections were treated before staining: a. Paraffin sections were dewaxed and hydrated: slices were baked at 60°C for 1 hour, and de-waxed in xylene for two times, each time for 5 minutes; then they were sequentially immersed in graded ethanol (anhydrous ethanol-anhydrous ethanol-95%-85%- 75% ethanol) and distilled water for 5 min each for hydration. b. Frozen sections and cell crawler sections (or crawler sections) were immersed in 0.01 MpH 7.4 PBS washed 3 times × 5 min. The tissues (or cells) were then covered with 0.1% TritonX-100 for 15 min of infiltration and washed 2 times × 5 min in 0.01 MpH7.4 PBS.

2) Antigen repair of paraffin sections: In most cases, paraffin tissue sections are suitable for high-pressure repair with citrate buffer. Preparation of repair solution: Add 10 ml of citrate buffer (IHC antigen repair solution, 100×) (Cat#: CW0128) to 1L of deionized water and mix well. Repair process: the repair solution was added into the pressure cooker, the section to be repaired was immersed in the repair solution (must be no more than the tissue), the lid of the pressure cooker was put on, heated until uniform vapor spraying, timed from the start of vapor spraying, after 1~2 minutes the pressure cooker was removed from the heat source and naturally cooled to room temperature, the section was taken out, rinsed with distilled water, and then rinsed with PBS (0.01 MpH7.4) for 2 times, each time for 3 minutes.

2. Incubate for 10 minutes at room temperature with appropriate amount of SolutionA white solution, i.e., endogenous peroxidase blocking solution, and rinse well with PBS.

3. Add appropriate amount of SolutionB white solution, i.e., closed with normal sheep serum working solution, incubate for 10 minutes at room temperature and shake dry.

4. Add appropriate amount of primary antibody working solution (commercialized ready-to-use antibody or concentrated antibody diluted in appropriate proportion), incubate according to the requirements of the experiment, and then fully rinse with PBS.

5. Add appropriate amount of SolutionC yellow solution, i.e. biotin-labeled sheep anti-rabbit/mouse secondary antibody working solution, incubate for 10 minutes at room temperature, and rinse well with PBS.

6. Add appropriate amount of SolutionD red solution, i.e., HRP-labeled streptavidin, incubate for 10 minutes at room temperature and rinse well with PBS.

7. Preparation of DAB color working solution: according to the required amount, mix DAB-A and DAB-B at a volume ratio of 1:19, that is, DAB color working solution. You can also choose to add 1 drop (about 50 μl) of reagent A per ml of reagent B and mix well.

8. Color development: add the appropriate amount of DAB color working solution in the need to develop the color of the tissue sections or cells on the crawl can be developed, the time for color development is generally 1-5 minutes. Observe under the microscope to control the color development time, when the best color development effect, tap water rinse to terminate the color development. After color development, the sections are re-stained, dehydrated and transparent, and can be stored for a long time after sealing.