Technical Data Sheet

Antibody Diluent for Frozen Sections

#62713-01, 62714-01, 62715-01

General Staining Protocol for Frozen Sections

Please read the entire procedure before starting the staining procedure. Perform all incubations in a humid chamber and do not allow sections to dry out. Isotype and system controls should also be run and must be matched to the isotype of each primary antibody to be tested.

Materials Needed

- Phosphate Buffered Saline (PBS)
- H_2O_2 Solution
- Antibody Diluent for IHC
- Streptravidin-Horseradish Peroxidase
- DAB Substrate Kit
- Hematoxylin
- Bluing Reagent
- Graded Alcohols
- Xylene

For your convenience, our Ig HRP detection kits can be used to perform the immunohistochemical staining. The Anti-Hamster Ig, Anti-Mouse Ig, and Anti-Rat Ig HRP detection kits are also available, and include Biotinylated Secondary Antibody, Antibody Diluent Buffer, Streptavidin-HRP, DAB Buffer, and DAB Chromogen.

Instructions

1. Label slides with a solvent-resistant pen and demarcate the tissue if required.
2. Rinse slides three (3) times in PBS to remove the tissue-freezing matrix.
3. Block endogenous peroxidase activity by incubating the slides in 0.3% H_2O_2 solution in PBS for 10 minutes.
4. Rinse slides three (3) times in PBS, 2 minutes each time.
5. Block non-specific binding by incubating with blocking buffer (10% serum from host species of secondary antibody diluted in PBS or 10% FBS in PBS) for 30-60 minutes at RT in a humidified chamber.
6. Dilute the primary antibody in the Antibody diluent for IHC. Alternatively, a buffered solution with a source of protein can be used as antibody diluent. Apply the diluted antibody to the tissue sections on the slide. Incubate for 1 hour at RT in a humidified chamber.
7. Rinse slides three (3) times in PBS, 2 minutes each time.
8. Dilute the biotinylated secondary antibody in the Antibody diluent for IHC. Alternatively, a buffered solution with a source of protein can be used as antibody diluent. Apply to the tissue sections on the slide and incubate for 30 minutes at RT.
9. Rinse slides three (3) times in PBS, 2 minutes each time.
10. Apply the Streptravidin-Horseradish Peroxidase pre-diluted to the tissue sections on the slide and incubate for 30 minutes at RT.
11. Rinse slides three (3) times in PBS, 2 times each.
12. Prepare DAB substrate solution by adding 1 drop of DAB chromagen to every 1 ml of DAB buffer.

WARNING: DAB is a suspect carcinogen. Handle with care. Wear gloves, a lab coat, and eye protection.

13. Drain PBS from slides and apply the DAB substrate solution. Allow slides to incubate for five (5) minutes or until the desired color intensity is reached.
14. Wash three (3) times in water, 2 minutes each time.
15. Counterstain slides:
    1. Dip twice in Hematoxylin.
    2. Rinse thoroughly in water.
    3. Dip twice in Bluing reagent or dilute ammonia water.
    4. Rinse thoroughly in water.
16. Dehydrate through 4 changes of alcohol (95%, 95%, 100%, and 100%). Clear in 3 changes of xylene (or xylene substitute) and coverslip using mounting solution.