Antigen Retrieval Protocol

Formalin fixation forms protein cross-links that can mask the antigenic sites in tissue specimens, thereby creating weak or false negative staining for immunohistochemical detection of certain proteins. There are currently several antigen retrieval methods commonly used and the protocols below are only intended as a general guide.

Heat Induced Epitope Retrieval (HIER)

Citrate Buffer (10mM Citric Acid, 0.05% Tween 20, pH 6.0):

Citric acid (anhydrous) ---------- 1.92 g

Distilled water ---------- 1000 ml

Mix to dissolve. Adjust pH to 6.0 w/ 1N NaOH and add 0.5 ml of Tween 20, mix well.

Pre-heat steamer or water bath with staining dish containing Sodium Citrate Buffer or Citrate Buffer until temperature reaches 95-100 degrees Celcius. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes. Remove the staining dish to room temperature and allow the slides to cool for 20 minutes before proceeding with normal staining procedure.

Tris Buffered Saline (TBS) (0.05M TBS, 0.05% Tween 20, pH 9.0):

Tris ---------- -6.1 g

Sodium Chloride ---------- 8.8 g

Distilled water ---------- 1000 ml

Mix to dissolve. Adjust pH to 9.0 using concentrated HCl and then add 0.5 ml of Tween 20 and mix well.

Pre-heat steamer or water bath with staining dish containing Sodium Citrate Buffer or Citrate Buffer until temperature reaches 95-100 degrees Celsius. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes. Remove the staining dish to room temperature and allow the slides to cool for 20 minutes before proceeding with normal staining procedure.

EDTA Buffer (1mM EDTA, 0.05% Tween 20, pH 8.0):

EDTA (Sigma, Cat# E-5134) ----- -------0.37 g

Distilled water ---------- 1000 ml

Mix to dissolve. Adjust pH to 8.0 using 1N NaOH. Then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4 C for longer storage.

Note: This buffer works excellent for many antibodies, but it often gives high background (maybe due to endogenous biotin revealed after this pretreatment). So primary antibody can often be highly diluted. It is very useful for low affinity antibodies or when tissue antigens are not intense.

Procedure:

1. Deparaffinize sections in 2 changes of xylene, 5 minutes each.
2. Hydrate in 2 changes of 100% ethanol for 3 minutes each, 95% and 80% ethanol for 1 minute each. Then rinse in distilled water.
3. Pre-heat steamer or water bath with staining dish containing EDTA buffer until temperatures reaches 95-100 degrees Celcius.
4. Immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20-40 minutes (optimal incubation time should be determined by user).
5. Turn of steamer or water bath and remove the staining dish to room temperature and allow the slides to cool for 20 minutes.
6. Rinse sections in was or 2 x 2 minutes.
7. Block sections with normal serum blocking solution for 30 minutes.
8. Perform avidin/blocking if necessary.
9. Incubate sections with primary antibody at appropriate dilution in antibody dilution buffer for 1 hour at room temperature or overnight at 4 degrees Celcius.

10. Rinse sections with wash buffer for 2 x 2 minutes.

11. Block sections with peroxidase blocking buffer for 10 minutes.

12. Rinse with wash buffer for 3 x 2 minutes.

13. Proceed with standard staining protocol.

Note: Microwave or pressure cooker can be used as alternative heating source to replace steamer or water bath.

Proteolytic Induced Epitope Retrieval (PIER)

Trypsin Working Solution (0.05%):

Trypsin stock solution (0.5%) ---------- 1 ml

Calcium chloride stock solution 1% --------- 1 ml

Distilled Water ----------8 ml

Adjust pH to 7.8 with 1N NaOH.

Cover sections with trypsin working solution and incubate for 10-20 minutes at 37 degrees Celsius in humidified chamber (optimal incubation time may vary depending on tissue type and degree of fixation, and should be determined by user). Allow sections to cool at room temperature for 10 minutes.

Proteinase K Working Solution (1x, 20 ug/ml):

Proteinase K Stock Solution (20X) ---------- 1 ml

TE Buffer, pH8.0 ---------- 19 ml

Mix well.

Cover sections with ProteinaseK working solution and incubate for 10-20 minutes at 37 degrees Celcius in humidified chamber (optimal incubation time may vary depending on tissue type and degree of fixation, and should be determined by user). Allow sections to cool at room temperature for 10 minutes.

Antigen retrieval for frozen sections

1% Sodium Dodecyl Sulfate (SDS) in PBS:

SDS ---------- 1 g

0.01M PBS (pH 7.4) ---------- 100 ml

Mix to dissolve.

Rinse sections three times for 5 min each in PBS. Cover sections with 1% SDS solution and incubate for 5 minutes at room temperature. Rinse 3 X 5 min each in PBS. (It is important to wash sections well). Proceed with staining as normal.

En Bloc antigen retrieval:

Procedure:

1. Fix tissue with buffered 4% PFA. The tissue blocks should be cut to a proper size (e.g. slides 3-5 mm thick).

2. Immerse the tissue blocks in Citrate Buffer at 4 degrees Celsius overnight.

3. Place the tissue blocks at 95-100 degrees Celsius for 3-5 minutes.

4. Immediately place the tissue blocks in cold 30% sucrose in PBS and incubate at 4 degrees Celsius until the blocks sink.

5. Immerse the tissue blocks in an embedding medium and freeze quickly with crushed dry ice. The frozen tissue blocks can now be stored at -80 degrees Celsius and process as normal.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.