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NB100-56733 Protocol

Immunohistochemistry (Paraffin) Protocol

Langerin/CD207 Antibody: https://www.novusbio.com/products/langerin-cd207-antibody_nb100-56733
DeParaffinization

Materials Required
- Tissue array slide - 100%, 95%, and 75% ethanol
- Xylene

Method

1. Incubate in a dry oven at 62C for 1 hour. Slides should be maintained in a vertical orientation to allow complete removal of the paraffin.
2. Dewax slides in xylene for 5 x 4 minutes.
3. Hydrate slides in 100%, 95%, and 75% ethanol for 2 x 3 minutes each.
4. Immerse slides in tap water for 5 minutes.

Suggested Antigen Retrieval Protocol

The following procedure are a suggestion only. Other protocols can be used on the array slides.

Materials Required
- Tissue array slide - Phosphate buffered saline (pH 7.6)
- Citrate buffer (0.01 M, pH 6.0) - Microwave oven (700 W)

Method 1 (Microwave)

1. Immerse slides into citrate buffer (0.01 M, pH 6.0).
2. Microwave (700 W or high) for 5 min, add citrate buffer if necessary.
3. Microwave (medium) for 5 min, add citrate buffer if necessary.
4. Microwave (low) for 5 min.
5. Immerse in cold PBS.

Method 2 (Autoclave/Pressure Cooker)

1. Immerse slides in citrate buffer.
2. Incubate in a pressure cooker for 2 min at 95C.
3. Cool to room temperature.
4. Wash slides in PBS for 3 x 5 min.

Method 3 (Enzyme treatment)

1. Incubate slides with pronase [0.05% (w/v) in PBS] or trypsin [0.05% (v/v) in PBS] or pepsin [0.05% (v/v) in 2 N HCl] at 37oC (incubation time should be adjusted according to the antibody).
2. Wash slides in PBS for 3 x 5 min.

Method 4 (Hot bath)

1. Heat citrate buffer (1mM EDTA, pH8.0 or 0.01M sodium citrate buffer, pH6.0) to about 950C.
2. Place slides in the buffer for 10-15 min.

Immunostain

Materials Required
1. Slides

2. Phosphate buffered saline (pH 7.6)
3. Hydrogen peroxide
4. Primary antibody
5. Blocking serum (normal serum)
6. Biotinylated secondary antibody
7. ABC reagent (6, 7, and 8 are included in Vectastain Elite ABC kit)
8. Diaminobenzidine
9. Meyer's hematoxylin
10.Permount

Method

1. Incubate in a dry oven at 62C for 1 hour. Slides should be maintained in a vertical orientation to allow complete removal of the paraffin.
2. Dewax slides in xylene for 5 x 4 minutes.
3. Hydrate slides in 100%, 95%, and 75% ethanol for 2 x 3 minutes each.
4. Immerse slides in tap water for 5 minutes
5. Antigen retrieval method (optional).
6. Quenching of endogenous peroxidase (optional)
a.immerse slides in 3% hydrogen peroxide solution for 6 minutes.
b.wash slides in PBS for 3 x 5 minutes.
7. Incubate slides with blocking serum (1:50) for 30 min.*
8. Blot excess serum from section, and incubate with primary Ab. Suggested incubation time may vary between antibodies:
mAb 2 hours at room temperature or overnight at 4C.
pAb: 1 ~ 1.5 hours at room temperature.
9. Wash slides in PBS for 3 x 5 minutes.
10.Incubate slides with biotin-conjugated secondary Ab for 30 min.*
11.Wash slides in PBS for 3 x 5 minutes.
12.Incubate slides with Avidin-Biotin Complexes for 30 min.*
13.Wash slides in PBS for 3 x 5 minutes.
14.Incubate slides in fresh DAB solution for 2 minutes. (We use DAB solution in Vector DAB/Ni substrate kit).**
15.Stop the reaction by washing in tap water.
16.Counterstain in Meyer's hematoxylin for 10 seconds.
17.Dehydrates slides in 75%, 80%, 95% and 100% ethanol
18.Clear slides in xylene 4 X 5 minutes.
19.Mount cover slide with Permount.

* Blocking serum, secondary antibody and avidin-biotin-peroxidase complexes are included in most of the immunostaining kit. Our lab uses the ABC kit from Vector Lab (Vectastain Elite ABC kit).
** We use DAB solution in Vector/DAB/Ni substrate kit (Vector Labs., Cat. SK-4100).