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Fluorescence immunohistochemistry

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Note that any protocol involving animals should be reviewed and approved by your Institutional Animal Care and Use Committee (IACUC) before use.

Abstract

This protocol describes the procedure for immunohistochemistry detected by fluorescence used in the Seifert Lab. It assumes a starting sample of a paraffin-embedded tissue section.

Protocol materials

- Xylene **Fisher Scientific** **Catalog #**X3P-1GAL
- 100% Ethanol
- 100% Ethanol
- Tris-buffered saline (TBS), 1x solution **Fisher Scientific** **Catalog #**BP24721
- Hoechst 33342 **Cell Signaling Technology** **Catalog #**4082
- Prolong Gold **Thermo Fisher Scientific** **Catalog #**P36930

Troubleshooting

Day 1

1 Deparaffinize slides to 100% Ethanol by a series of:

- 2 washes of 00:05:00 each with 2X
  Xylene **Fisher Scientific Catalog #**X3P-1GAL
- 2 washes of 00:02:00 each with 100% Ethanol

2 Rehydrate to ddH_2O by a series of:

- 00:03:00 wash with 90% Ethanol
- 00:01:00 wash with 70% Ethanol
- 2 washes of 00:01:00 each with ddH_2O

3 *(SKIP IF FROZEN)*
Perform retrieval, optimized for antigen and treatment

Note

For a first run, can try heat + citrate buffer pH 6, heat + DAKO high pH 9, proteinase K
*After heat, transfer directly to ddH_2O to cool before proceeding to first wash with TBS
**For matrix proteins, try 2' proteinase K

4 Wash for 00:05:00 with
Tris-buffered saline (TBS), 1x solution **Fisher Scientific Catalog #**BP24721

5 Block for at least 00:30:00 at Room temperature in [M] 15 µL/mL appropriate serum in TBS

Note

The serum is typically whatever the secondary antibody was raised in; for multiple labeling, use a 50:50 mixture of the two sera

6 *(SKIP IF NOT AMPLIFYING SECONDARY ANTIBODY)*

Avidin/Biotin Block

6.1 Block with avidin for 00:15:00 at Room temperature

6.2 Wash for 00:05:00 in TBS

6.3 Block with biotin for 00:15:00 at Room temperature

6.4 Wash for 00:05:00 in TBS

7 Incubate with primary antibody or control in [M] 15 µL/mL serum in TBS Overnight at 4 °C in a humidity box

Note

The preferred control is IgG at the same concentration as the primary antibody, but otherwise just
leave out the primary antibody

Day 2

8 Wash for 00:05:00 in TBS

9 Incubate with secondary antibody (biotinylated secondary to the species of the primary) at 1:400 in [M] 15 µL/mL serum in TBS for 00:30:00 at Room temperature

Note

1:400 is usually a good dilution, but this can be reduced if background is too high

10 Wash for 00:05:00 in TBS

11 Incubate with desired AlexaFluor-conjugated secondary antibody at 1:400 in TBS (without serum) for 00:30:00 at Room temperature

12 Wash for 00:05:00 in TBS

13 Wash for 00:05:00 in ddH_2O

14 Incubate with [M] 1 µg/mL
Hoechst 33342 **Cell Signaling Technology Catalog #**4082 for 00:05:00 at Room temperature

15 Wash with ddH_2O

16 Stand slide at an angle and allow to drip-dry for a few minutes

17 Cover slip

Note

If using Prolong Gold **Thermo Fisher Scientific Catalog #**P36930 can cover slip when a bit wet