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Double immunofluorescence – Paragraffin-embedded sections

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We use this protocol and it's working

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Abstract

In order to be able to examine the co-distribution of two (or more) different antigens in the same sample, a double immunofluorescence procedure can be carried out. Primary antibodies raised in different species can be used either in
parallel (in a mixture) or in a sequential way.

Preparation of slides and samples

1 1.1. Deparaffinize sections in xylene 2×5 min.
1.2. Hydrate with 100% ethanol 2×3 min.
1.3. Hydrate with 95% ethanol 1 min.
1.4. Rinse in distilled water

2. Fixation

2 2.1 Fix the samples either in ice-cold methanol, acetone (1-10 min) or in 3-4% paraformaldehyde in PBS pH 7.4 for 15 min at room temperature.
2.2 Wash the samples twice with ice cold PBS.

3. Permeabilization

3 3.1 Incubate the samples for 10 min with PBS containing 0.25% Triton X-100
3.2 Wash cells in PBS three times for 5 mins.

4. Blocking and simultaneous incubation

4 4.1. Incubate tissues with 1% BSA in PBST for 30 min to block unspecific binding of the antibodies
4.2. Incubate tissues in **the mixture of two primary antibodies** in 1% BSA in PBST in a humidified chamber for overnight at 4^{o} C.
4.3. Decant the mixture solution and wash the cells three times in PBS, 5 mins each wash.
4.4. Incubate cells with **the mixture of two secondary antibodies** which are raised in different species in 1% BSA for 1 hr at room temperature in dark.
4.5. Decant the mixture of the secondary antibody solution and wash three times with PBS for 5 min each in dark.

5. Counter staining

5 5.1. Rinse with PBS.
5.2 Incubate tissues on 0.1-1 µg/ml DAPI for 1 min.

6. Mounting

6 6.1. Seal coverslip with nail polish to prevent drying and movement under microscope.

6.2. Store in dark at -20°C or 4°C.