Antigen retrieval on histological sections

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Abstract

Detailed protocol for chemical +/- heat antigen retrieval on sectioned tissue to increase reactivity and specificity of antibody recognition

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Protocol

Poly-L-lysine pretreatment of slides

Step 1.

Dilute Sigma P8920 Poly-L-Lysine solution (0.1% w/v) 1:10 in distilled water to make working solution. Make up fresh each time. One slide chamber holds 200 ml of solution and can be used for four consecutive racks of slides (= 100 slides total). If dipping more than 100 in one session, make up another batch of solution.

Slides: Any charged slides with opaque frosted glass are ok to use. Beware the semi-transparent frosted glass—they are really hard to work with.

Fill 25-slot slide holders with slides and process ‘assembly-line’ style:

*Clean slides in acetone for 5 minutes

*Air dry slides at an angle (leaning on a tube rack or other suitable support) on paper towels until all droplets of acetone are evaporated.

*Dip slides in 1:10 diluted Poly-L-Lysine solution in a plastic slide chamber for 15 minutes

*Air-dry slides at an angle on paper towels for a few minutes until big drops are gone

*Bake the slides for 1 hour at 55°C.

Once dry, transfer slides to racked slide boxes and store at -20°C until use

3'-aminopropyltriethoxysilane (APTES) pretreatment of slides

Step 2.

APTES coating is stickier than Poly-L-lysine and thus may be more suitable for some tissues.

Dilute Sigma A3648 APTES solution 1:50 in acetone for a 2% solution. This is stable at room temp for 8 hours.

Fill 25-slot slide holders with slides and process 'assembly-line' style:

*Clean slides in acetone for 5 minutes

*Air dry racked slides at an angle until acetone evaporates

*Dip slides in 2% APTES solution for 2 minutes

*Drain off solution, then put rack through 2 washes of dH20 for 2 minutes each

*Air dry until big droplets are gone

*Bake the slides for 1 hour at 55°C

Once dry, transfer slides to racked slide boxes and store at room temperature until use.

Sodium Citrate Antigen Retrieval using a Pressure Cooker

Step 3.

*This protocol is suitable for cryo or paraffin sections

*Rehydrate sections in PBST, 2 times 10’ at RT.

- put slides in a plastic Coplin Jar (glass can shatter in the pressure cooker)

- fill with 10 mM sodium citrate (pH 8.5)

- Add 100ml dH2O to cooker (just enough to cover the bottom & generate steam) and set pressure cooker timer for 10 minutes

- once cooking time has concluded, release steam **very** gradually with small incremental movements of the valve—avoid bringing the contents to a boil, which will occur if steam is released too rapidly

- Remove coplin jar from cooker and cool at RT until the solution is at or below 37°C. Coplin jar can be placed at 4°C if you’re pressed for time.

-Transfer slides to PBST washes and proceed with your favorite antibody labeling protocol.

Alternative Antigen Retrieval Methods

Step 4.

Citrate + Heat AR has the highest rate of success on PFA fixed paraffin or cryosectioned tissue. Alternative methods to try if unsuccessful include EDTA + heat and Trypsin treatment

For EDTA:

- after tissue hydration washes, put slides in a plastic Coplin Jar

- fill up with 0.8-1 mM EDTA solution

- heat for 10’ in pressure cooker as described in Step 3, above

- cool as described

For Trypsin:

Apply undiluted Trypsin to individual slides for 10-20 minutes at RT, depending on sturdiness of tissue; rinse thoroughly.

Neither of the above approaches worked well in my hands on cryosectioned tissue, but are reported to have better outcomes on paraffin sections.

Warnings

Make sure chemical waste is disposed of according to on site EHS guidelines