Smith Lab
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HIF-1α IHC Protocol (with SuperPicture kit)

Materials

Citrisolve
Ethanol (100%, 95%, 70%)
dH2O
Slide marker (PAP pen)
TBS (make from tablet)
TBS+Tween (make from tablet)
Dako Antibody Diluent
Hydrogen peroxide (3%) (dilute in dH2O from 30%)
Background Buster Blocking Agent
Antigen Retrieval Buffer
SuperPicture Kit Reagents, broad spectrum
Primary Antibody (Abcam Ab1 Anti HIF-1α)

- Use fresh ethanols
- Check expiration dates of all reagents.
- Avoid multiple freeze-thaws of antibodies.
- Use disposable transfer pipettes and horizontal incubation chamber for all steps except rehydration.

Procedure

<u>Deparaffinize and rehydrate</u>

- 5 minutes Citrisolve (x2)
- 5 minutes 100% EtOH (x2)
- 5 minutes 95% EtOH
- 5 minutes 70% EtOH
- 5 minutes dH2O

<u>Preparation for Primary Ab</u>

- Demarcate around section with slide marker
- 3 minute TBS wash (x3)
- Perform Antigen Retreival (careful sections don't fall off during this step):
    - Place glass coplin jar in water bath (submersed a few inches)
    - Fill jar with antigen retrieval buffer, and place thermometer in jar.
    - Heat retrieval buffer to 95-100°C
    - Place slides in jar and incubate for 10 mins
    - Remove slide and wash gently in dH2O.
- 3 minute TBS wash (x3)
- 10 minutes 3% H2O2 (for quenching endogenous peroxidase)
- 3 minute TBS wash (x3)
- 30 minutes blocking using Background Buster

<u>Primary Ab</u>

- Dilute primary Ab in blocking solution at desired concentration
- Include positive and negative controls as appropriate
- Incubate overnight in humidified chamber at 4 degrees

<u>Secondary Ab</u>

- 3 minute TBS+Tween wash (x4)
- 10 minute incubation with secondary Ab at room temperature
- 3 minute TBS+Tween wash (x4)
- 3 minute dH2O wash

Developing

- Create developing solution with 1 mL dH2O, 50 uL B1 reagent, 100 uL B2 reagent, 50 uL B3 reagent. Solution should be made within 30 minutes of use and protected from the light.
- Add developing solution and incubate in the dark for as long as necessary before background develops. Usually around 1-4 minutes. Check on microscope and continue for longer if necessary.
- Rinse 3-4x with dH2O to stop development
- Coverslip with aqueous mounting medium and seal with nail varnish.

Antigen Retrieval Buffer:

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

Tri-sodium citrate (dihydrate) --------- 2.94 g

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

Mix to dissolve. Adjust pH to 6.0 with 1N HCl and 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.

Primary Antibody Optimization

To find the optimal antibody dilution, run the protocol with a positive control, and test several different antibody dilutions (e.g. 1:50, 1:100, 1:200, 1:500, 1:1000). 1:200 is about average.

Positive Controls

Find a tissue know to highly express the antigen of interest.

Negative Control

Best: Replace primary antibody with isotype matched control antibody

Second best: Leave out primary antibody (just incubate with diluent)