

NB100-360 Protocol

Immunohistochemistry protocol for ATP7b Antibody (NB100-360)

[[URL:https://www.novusbio.com/products/atp7b-antibody_nb100-360]][[Caption:ATP7b Antibody]]
Immunohistochemistry Procedure

Cell Preparation (At least 108 cells were used per block)

1. Harvesting cells:

- A. Trypsinization
- B. 15 minute centrifugation at 2,500 RPM
- C. PBS rinse
- D. 15 minute centrifugation at 2,500 RPM

2. Suspend cells in 10 ml of 10% formaldehyde in PBS, overnight @ RT.

3. Centrifuge cells at 2,500 RPM for 10 minutes.

4. Resuspend cells in 10 ml of 70% ethanol.

5. Centrifuge cells at 2,500 RPM and taken into 70% ethanol.

Cell Staining

1. Ribbon Thickness: 5 um
2. Deparaffination Agent: Xylin
3. Hydration: Ethanol in PBS
4. Antigen Retrieval: 10 minute microwave retrieval in citrate buffer; 20 minute cooling
5. Blocking:

A. endogeneous peroxidase: 0.3% H₂O₂ in PBS for 10 minutes

B. endogeneous protein: 1% BSA for 20 minutes

6. Primary antibody, polyclonal anti-ATP7b (NB 100-360): 1:500, overnight @ 4 degrees Celcius

7. Secondary antibody, anti-rabbit (HRP): (dilute per manufacturer recommendation), 30 minutes @ RT

8. Wash 3x 15 minutes

9. Chromogen: AEC

10. Counterstain: Mayers hematoxylin

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.