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NB100-125 Protocol

Immunocytochemistry/Immunofluorescence Protocol for PER2 Antibody (NB100-125)

Immunocytochemistry Protocol

Culture cells to appropriate density on suitable glass coverslips in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 5-10 minutes.
2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.
3. Remove the permeabilization buffer and add wash buffer (i.e. PBS or PBS with 0.1% Tween-20). Be sure to not let the specimen dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20C methanol for 10 min at room temperature. Remove the methanol and rehydrate in PBS for 10 min before proceeding.
5. To block nonspecific antibody binding incubate in 10% normal goat serum for 1 hour at room temperature.
6. Add primary antibody at appropriate dilution and incubate at room temperature for 1 hour or at 4C overnight.
7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.
8. Add secondary antibody at the appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei can be staining with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.
11. Cells can now be viewed with a fluorescence microscope.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.