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NB100-1869 Protocol

Immunocytochemistry/Immunofluorescence protocol for SLC1A3 antibody (NB100-1869)

[[URL:https://www.novusbio.com/products/eaat1-glast-1-slc1a3-antibody_nb100-1869]][[Caption:SLC1A3 antibody]]

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

1. Remove culture medium and gently add 10% formalin to cover the cells. 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 (PBS or PBS with 0.1% Tween-20). Be sure to not let the cells dry out. Gently wash three times for 10 minutes.
4. Alternatively, cells can be fixed with -20 degrees C methanol for 10 minutes. Remove the methanol and rehydrate in PBS for 10 minutes before proceeding.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum for 60 minutes at room temperature.
6. Add primary antibody at the appropriate dilution and incubate at room temperature for 60 minutes or at 4 degrees C 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 60 minutes at room temperature.
9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.
10. Nuclei (DNA) can be stained 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.