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## **NB110-39113 Protocol**

Immunocytochemistry/Immunofluorescence Protocol for Glut1 Antibody (NB110-39113)

Immunocytochemistry Protocol

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

- 1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
- 2. Remove the formalin and wash the cells in PBS.
- 3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
- 4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
- 5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
- 6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
- 7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
- 8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
- 9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
- 10. Counter stain DNA with DAPi if required.