

## NBP1-49533 Protocol

### Immunocytochemistry/Immunofluorescence Protocol for Glucose Transporter GLUT4 Antibody (NBP1-49533)

#### Immunocytochemistry Protocol

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

1. Pull off culture medium with and add 10% formalin to the dish. Fix at room temperature for 30 minutes..
2. Take off the formalin and add ice cold methanol (kept in well sealed bottle in -20C). Incubate for 5-10 minutes.
3. Take off methanol and add PBS (You can add 0.1% Tween-20 to PBS used here and all subsequent steps), be sure to not let the specimen dry out. Wash 3 times 10 minutes before proceeding to blocking step.
4. To block nonspecific antibody binding incubate in 10% normal goat serum for a minimum of 1 hr at room temp. Cells can also block overnight at 4C for this step.
5. Add primary antibody at appropriate dilution and incubate at room temp for 2 hrs or overnight at room temp.
6. Remove primary antibody and replace with PBS. Wash 3 x 10 min in PBS.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hr at room temperature
8. Remove antibody and replace with PBS, wash 1 x 10 min in PBS. Add Hoechst 33258 to PBS at 1:25,000 and incubate for 10 min. Wash a third time with PBS for 10 min (total of 3X10min PBS washes).
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide and parafilm. Cells can also be coverslipped using Fluoromount. If storing coverslip be sure to seal the edges with clear nail polish.

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