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## NBP2-22164 Protocol

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ICC/IF Protocol Specific for NBP2-22164: DAT1 Antibody (mAb16)

[[URL:https://www.novusbio.com/products/slc6a3-dat1-antibody-mab16\_nbp2-22164]][[Caption:SLC6A3/DAT1 Antibody (mAb16)]]
Immunocytochemistry Protocol

Culture cells to appropriate density 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 30 minutes.
- Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
- Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
- 4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
- 5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
- 6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
- 7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
- 8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
- 9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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