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NB100-56511 Protocol

Immunocytochemistry/ Immunofluorescence Protocol for MyoD Antibody (NB100-56511)

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 4% paraformaldehyde to the dish and fix at room temperature for 10 minutes.
2. Remove the paraformaldehyde and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 2 min.
4. Remove the permeabilization buffer and wash three times for 5 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 5 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 5 minutes each.
10. Counter stain DNA with DAPI if required.