General Protocol for ICC Staining of Live Cells

Overview

The following procedure is a general protocol for immunocytochemistry (ICC) of live cells in culture. All steps should be performed in a sterile working environment using fresh culture medium for the continuation of the cell culture. Only open the antibody in the biological safety cabinet to prevent possible contamination.

Required Materials

- Cell culture medium

ICC Procedure

1. Culture the cells at 37°C and 5% CO_2 to the desired confluency.
2. Dilute the primary antibody to a final concentration of 2.5 to 5 µg/mL in fresh cell culture medium.
3. Aspirate the medium from the cells and add the diluted primary antibody to the live cells.
4. Incubate for 30 minutes at 37°C and 5% CO_2.
5. Aspirate the primary antibody and wash the cells gently 2 times with cell culture medium.
6. Add fresh cell culture medium and examine under a fluorescent microscope using the appropriate filters.

Note: Do not allow cultures to be kept outside of the incubator for extended periods of time to avoid drastic temperature changes and the possibility of contamination.

7. Return the plate to the incubator to continue culture at 37°C and 5% CO_2.

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