Transferrin Uptake using Fluorescence Microscopy

Materials

Pre-warmed serum-free DMEM medium
Alexa dye-conjugated transferring (10ug/mL; Life Technologies)
BSA
Glycine buffer (0.1 M Glycine pH 2.5; 150 mM NaCl)
PFA

1. Culture cells on coverslips to 60-70% confluence.

2. Starve cells for 30 mins at 37℃ in pre-warmed serum-free DMEM medium.

3. Incubate cells for 10 min at 37℃ with Alexa dye-conjugated transferrin diluted in prewarmed DMEM and 0.1% BSA.

4. Wash cells to remove extracellular transferrin with ice-cold PBS.

5. This is followed by a 5-min incubation with cold glycine buffer [0.1 M glycine (pH 2.5), 150 mM NaCl].

6. Wash cells with cold PBS for two more times. Cells are then fixed with 4% PFA at RT for 20min for immunofluorescence.

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