Sugden Lab.

Transient Transfection by DEAE/Chloroquine

1. The day before transfection plate cells into 6 cm dish so that they are 70~90% confluent the day of transfection.

2. Dilute DNA into 1 ml DEAE transfection mixture*, mix gently, and incubate at 37˚C until transfection. Avoid oxidation of the transfection mixture. Incubate the chloroquin media at 37˚C.

3. Remove the medium from the culture dish and wash it twice with 5 ml washing medium added gently to side of the plate.

4. Remove the washing buffer from the plate and add the DEAR transfected mixture and allow mixture to cover entire plate.

5. Incubate the plate for 30~45 min at 37˚C in a CO_2 incubator. Every 10 min during the incubation rock plate to redistribute mixture over plate.

6. In dark food add chloroquine component into pre-incubated chloroquine medium and shade the tube by foil.

7. Remove the transfection mixture from the cells, wash plates twice with 5 ml washing medium and then add 10 ml of chrolouquine mixture.

8. Incubate the cells for 4 h and replace medium of plate with 10 ml feeding medium and incubate for 24~72 h at 37˚C in a CO_2 incubator.

1. Transfection mixture:

    DMEM/high glucose
    20 mM HEPES pH 7.25
    500 µg/ml DEAE-dextran

2. Chloroquine medium:

    DMEM/high glucose
    100 µM Chloroquine
    2% FBS

3. Washing medium

    DMEM/high glucose
    20 mM HEPES pH 7.24

4. Feeding medium

    DMEM/high glucose
    2% FBS
    4 U/ml Penicillin
    40 µg/ml streptomycin

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