ABCEPTA CUSTOM SERVICES:

Transfection Protocol

Materials

- Cell line cultured in the appropriate growth medium
- Plasmid DNA
- Lipofectamine
- Basic Medium (without serum)
- Appropriate cell culture plates and supplies

Procedure

1. Adherent cells: Plate 0.5-2 × 10^5 cells in 500 uL of growth medium without antibiotics at the day before transfection, so that cells will be 90-95% confluent at the time of transfection.
   Suspension cells: Just plate 4-8 × 10^5 cells in 500 uL of growth medium without antibiotics.
2. For each transfection sample, prepare complexes as follows:
   a. Dilute DNA in 50 uL of Basic Medium. Mix gently.
   b. Mix Lipofectamine gently before use, then dilute the appropriate amount (DNA: Lipofect = 1:2.5) in 50 uL of Basic Medium. Incubate for 5 minutes at room temperature.
   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine (total volume = 100 uL). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).
   Note: All the steps should be finished with in 25 minutes.
3. Add the 100 uL of complexes to each well containing cells and medium. Mix gently by shaking the plate left and right.
4. Incubate cells at 37 ℃ in a CO_2 incubator for 18-48 hours before testing for protein expression. Medium may be changed after 4-6 hours.
5. Stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) the following day.

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