

Transfecting Insect Cells

Edited 11/10/2017 by A.Palillo

Adapted from Thermo scientific published guidelines

1. Verify that the cells are in the log phase ($1.5-2.5 \times 10^6$ cells/ml) with greater than 95% viability.
2. Plate 2 mL of the cells from Step 1, diluted to $0.8-1.5 \times 10^6$ cells/mL, per well. Allow cells to attach for 30 minutes at 28°C. Remove the medium. Add 2.5 mL unsupplemented SF900 II SFM per well.
3. For each transfection sample, prepare complexes as follows:
 - a. Mix Cellfectin II before use (pipette up and down), and dilute 8 uL in 100 uL unsupplemented SF900 II SFM. Mix gently.
 - b. Dilute 1 uL (~1000ng) baculovirus DNA in 100 uL unsupplemented SF900 II SFM. Mix gently.
 - c. Combine the diluted DNA with diluted Cellfectin II (total volume ~210 uL). Mix gently and incubate for 15-30 minutes at room temperature.
4. Add ~210 uL transfection mixture (Step 3c) PER WELL dropwise onto the cells from Step 2. Incubate cells at 28°C for 4-24 hours.
5. Remove the transfection mixture and replace with 2.0 ml – 2.5 mL of supplemented SF900 II SFM (5% FBS, 1 % Anti-anti).
6. Incubate cells at 28°C for 5 days or until you see signs of viral infection.

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