REAGENT 007

PRODUCT DESCRIPTION:
Reagent 007 is a formulation for transfecting bacmid DNA into adherent Sf9 cells growing in serum-free medium

PACKAGE CONTENTS:
Cat# R007-0750 – 0.75ml
Cat# R007-1500 – 1.5 ml
Cat# R007-5000 - 5 ml
Cat# R007-P001 - 1 mg
Cat# R007-P010 - 10 mg

STORAGE CONDITIONS:
Store at +2-8°C for up to 9 months.
Avoid freezing/thawing cycles.
Lyophilized powder can be stored at -20°C for up to 1 year

ICSA

REAGENT 007

REQUIRED MATERIALS:
- Bacmid DNA
- Sterile deionized water
- Suitable serum-free medium
- Microcentrifuge tubes
- 6-well plates for transfection

We have found the following media to be suitable for use as a transfection medium with Reagent 007:

Ex-Cell 420 (Sigma-Aldrich, Cat # 14420C)
Sf900 II SFM (Gibco, Cat # 10902)
TNM-FH (Sigma-Aldrich, Cat # T3285)*

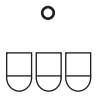
* Prepare cells in TNM-FH medium without serum for transfection and add serum or change medium after 2-4 hours of incubation

Supplementation of the transfection medium with additional Pluronic® F-68 or any other anti-clumping agent is not recommended as it may cause a dramatic decrease in transfection efficiency!

PROTOCOL

1. Seed cells
- Seed 8x10^5 cells in 2 ml on a 6-well plate

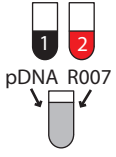
Step 1



- Let the cells attach for 0.5 to 1 hours at 28°C

2. Form complexes
- Prepare Reagent 007 Working Solution in an amount sufficient for all transfections (20 µl per reaction). To prepare a Working Solution, dilute the Stock Solution 5 times in deionized water: 1 volume Stock Solution + 4 volumes water. A Working Solution can be stored for further use at +4°C for up to one week.

Step 2



- Prepare bacmid solution in water into appropriate tubes containing 500 ng in 30 µl deionized water per reaction.

If initial stock of bacmid DNA is prepared in TE buffer (10 mM TrisHCl, 1 mM EDTA), the volume of TE in final volume of complex must not exceed 10%

- Add 20 µl of Reagent 007 Working Solution to the DNA solution and mix immediately by pipetting gently up and down 5-6 times.

Do not vortex the complex, it will decrease transfection efficiency!

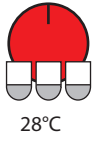
Step 3

3. Incubate at room temperature for 5-10 minutes.

Step 4

4. Add DNA/R007 complex by pipetting it drop-wise to the cells and mix with cell media by using gentle movements

Step 5



5. Incubate at 28°C for 3 days*

Step 6*

***6. When using cells that require presence of serum in medium,** incubate cells in serum-free medium for 2-4 hours after transfection and add 10% FBS or change medium. The incubation is continued at 28°C.

6-well format

Suitable medium	2 ml
Cells	8x10^5
bacmid DNA in water	500 ng in 30 µl
Reagent 007 Working Solution	20 µl
DNA/R007 complex final volume	50 µl

NOTES:
1) For further upscaling just increase all quantities and volumes proportionally
2) For transfection, always use cells in exponential growth phase with a viability >95%