



**This product is intended for research purposes only**

**CAUTION: Not intended for human or animal diagnostic or therapeutic uses.**

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**AcceloFectin™ Transfection Reagent**

**Product No.: 20010**

**Size: 500 µL**

**Product No.: 20020**

**Size: 1 mL (2x500 µL)**

**Store at 4°C**

**Description**

AcceloFectin Transfection Reagent is a polycationic formulation in membrane-filtered water that is suitable for transfection of DNA into insect cells. AcceloFectin has low cytotoxicity and can transfect Sf9 cells.

For further information on other cell types that can be transfected using AcceloFectin contact Technical Services at [info@paratechs.com](mailto:info@paratechs.com) or call (859) 317-9213.

**Guidelines for Transfection**

1. As a starting point form complexes using the amounts of DNA and AcceloFectin recommended in this manual (see page 2). Optimization with DNA preparations and cell types may be necessary.
2. AcceloFectin is a polycation suspension. Mix thoroughly through inversion of the tube 4-5 times.
3. Keep AcceloFectin sterile by practicing standard aseptic techniques used for insect tissue culture.
4. **Do not add antibiotics** to the media during transfection as this causes cell death.
5. Procedures are provided for the transfection of cells in a 6-well format. Optimization for other formats is necessary.

### **Transfecting Sf9 Cells with transient expression vectors**

Use this protocol for transfection of Sf9 cells in a **6-well plate format**. All amounts and volumes are given on a per well basis.

1. Seed  $1.5 \times 10^6$  Sf9 cells in 2 mL of Sf900 II SFM (Invitrogen™) containing **no antibiotics**. Allow cells to adhere for 0.5 to 1 hr.
2. **Preparation of transfection complexes:**
  - a. Dilute 1 µg of DNA in 1 mL of SF900 II SFM without antibiotics and mix by gentle inversion.
  - b. Add 4-8µL of AcceloFectin to the diluted DNA and mix by gentle inversion and allow to incubate for 15-30 minutes at room temperature.
3. Remove media from Sf9 cells and wash cells once with Sf900 II SFM without antibiotics. Remove wash medium. (This step is not necessary if no antibiotics were used in the original media.)
4. Add the transfection complexes from step 2b gently to the cells. Incubate the cells on a rocker and gently rock for 4-6 hrs.
5. Remove the transfection complexes and replace with 2 mL of Sf900 II SFM (This media may contain antibiotics). Incubate the cells at 27°C.
6. Test for transgene expression 24-72 hours post-transfection.

### **Transfecting Insect Cells with Baculovirus DNA**

Use this protocol to transfect Sf9 insect cells in a **6-well plate format**. All amounts and volumes are given on a per well basis.

1. Seed  $1 \times 10^6$  to  $1.5 \times 10^6$  Sf9 cells in 2 mL of Sf900 II SFM (Invitrogen) containing **no antibiotics**. Allow cells to adhere for 0.5 to 1 hr.
2. **Preparation of transfection complexes:**
  - a. Place 500 ng of linearized baculovirus DNA with 1-2 µg of baculovirus transfer vector in a sterile 1.5 mL eppendorf tube.
  - b. Add 1 mL of Sf900 II SFM without antibiotics and gently mix by inversion.
  - c. Add 8 µL of AcceloFectin to the diluted DNA (step 2b) and gently mix by inversion and allow to incubate for 15-30 minutes at room temperature.
3. Remove media from Sf9 cells and wash cells once with Sf900 II SFM without antibiotics. Remove wash medium. (This step is not necessary if no antibiotics were used in the original media.)
4. Add the transfection complexes from step 2b gently to the cells. Incubate the cells on a rocker and gently rock for 4-6 hrs.
5. Remove the transfection complexes and replace with 2 mL of Sf900 II SFM (this media may contain antibiotics). Incubate the cells at 27°C.
6. Assay for gene activity at 4 days post-transfection; harvest virus at 5 days post-transfection.

### **Optimizing Transfections**

To obtain the highest transfection efficiency with the least non-specific effects with Sf9 and other cell lines, optimization of transfection conditions may be necessary by varying cell density, DNA and AcceloFectin concentrations, and transfection incubation time.