








	<b>Package Contents</b>	<table><tr><th>Catalog Number</th><th>Size</th></tr><tr><td>10362-100</td><td>1 mL</td></tr><tr><td>10362-125</td><td>125 µL (sample size)</td></tr></table>	Catalog Number	Size	10362-100	1 mL	10362-125	125 µL (sample size)
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10362-100	1 mL							
10362-125	125 µL (sample size)							
	<b>Storage Conditions</b>	<ul style="list-style-type: none"><li>▪ Store at 4°C.</li><li>▪ Do not freeze.</li></ul>						
	<b>Required Materials</b>	<ul style="list-style-type: none"><li>▪ Sf9, Sf21, or High Five™ cells</li><li>▪ Grace's Insect Medium, Unsupplemented</li><li>▪ Sf-900™ II SFM or Sf-900™ III SFM (optional)</li><li>▪ PLUS™ Reagent (if using High Five™ Cells)</li><li>▪ Plasmid or Bacmid DNA</li></ul>						
	<b>Timing</b>	Preparation: 15–30 minutes Initial incubation: 15–35 minutes Incubation with mixture: 3–5 hours Incubation with growth medium: 24–72 hours						
	<b>Selection Guide</b>	<a href="#">Transfection Reagents</a> Go online to view related products.						
	<b>Product Description</b>	<ul style="list-style-type: none"><li>▪ Cellfectin™ II Reagent is a proprietary, cationic lipid formulation suitable for transfecting DNA into insect cells.</li><li>▪ Use this reagent to transfect Sf9, Sf21, and High Five™ cells in Bac-to-Bac™, BaculoDirect™, and InsectSelect™ Expression Systems or equivalent systems.</li></ul>						
	<b>Important Guidelines</b>	<ul style="list-style-type: none"><li>▪ Before use, mix this lipid reagent thoroughly by inverting 5–10 times.</li><li>▪ DNA-Cellfectin™ II complexes must be made in serum-free medium.</li><li>▪ During transfection, use 1.5% serum in medium.</li><li>▪ Do not add antibiotics to media during transfection.</li></ul>						
	<b>Online Resources</b>	Visit our <a href="#">product page</a> for additional information and protocols. For support, visit <a href="http://www.thermofisher.com/support">www.thermofisher.com/support</a> .						

## Protocol Outline

- Prepare cells and medium.
- Dilute lipid reagent.
- Dilute DNA.
- Combine DNA and lipid reagent.
- Add DNA-lipid complex to cells.
- Replace media.

## Cellfectin™ II Transfection Protocol

Transfection protocols for Sf9 and Sf21 cells are described in this manual. To obtain the best results, perform transfections using the appropriate protocol for your system and cell type.

-  See page 2 to view a procedure for transfecting Sf9 or Sf21 with Baculovirus DNA.
-  See page 3 to view a procedure for transfecting Sf9 or Sf21 with Plasmid DNA.

## Optimizing Transfections

Optimize transfection conditions by varying cell density, DNA, and Cellfectin™ II Reagent concentrations and transfection incubation time.

## Limited Product Warranty and Disclaimer Details

## Limited Use Label License

# Transfecting Sf9 or Sf21 Cells in Bac-to-Bac™ or BaculoDirect™ Systems

Use the following procedure to transfect Sf9 or Sf21 insect cells cultured in Grace's Insect Medium, Sf-900™ II SFM or Sf-900™ III SFM, Supplemented, containing 10% FBS in a 6-well format. All amounts are given on a per-well basis.

Timeline		Steps	Procedure Details
Day 1	1	Prepare plating medium and cells	At the time of transfection, ensure that you have $8 \times 10^5$ cells/well in 6-well format in medium containing 1.5% serum. a. Prepare 10 mL of plating medium by mixing 1.5 mL Grace's Insect Medium, Supplemented, containing 10% FBS (without antibiotics) and 8.5 mL Grace's Insect Medium, Unsupplemented (without FBS or antibiotics). a. Plate $8 \times 10^5$ of Sf9 or Sf21 cells per well in a 6-well plate. Allow the cells to attach for 15 minutes at room temperature in the hood. Remove the medium and replace with 2.5 mL of plating medium.
	2	Dilute Cellfectin™ II Reagent in Grace's Medium, Unsupplemented	a. Mix the Cellfectin™ II Reagent before use. b. Dilute 8 µL in 100 µL Grace's Medium, Unsupplemented. c. Vortex briefly to mix. <b>Note:</b> You may keep this mixture at room temperature for up to 30 minutes.
	3	Dilute DNA in Grace's Medium, Unsupplemented	<b>Bac-to-Bac™ System:</b> Dilute 1–3 µg of Baculovirus DNA into 100 µL of Grace's Insect Medium, Unsupplemented, and mix gently. <b>BaculoDirect™ System:</b> Dilute 10 µL of LR recombination reaction into 100 µL of Grace's Insect Medium, Unsupplemented, and mix gently.
Days 2–3	4	Add diluted DNA to diluted Cellfectin™ II Reagent and incubate	Combine the diluted DNA with diluted Cellfectin™ II Reagent (to a total volume of ~210 µL), and mix gently. Incubate for 15–30 minutes at room temperature.
	5	Add DNA-lipid complex to cells and incubate	Add ~210 µL DNA-lipid mixture dropwise onto the cells. Incubate at 27°C for 3–5 hours.
	6	Replace media and incubate	Three to five hours post-transfection, remove the transfection mixture and replace with 2 mL of complete growth medium (e.g., Grace's Insect Medium, Supplemented and 10% FBS). Incubate the cells at 27°C for 72 hours or until you see the signs of viral infection.

# Transfecting Sf9, Sf21 or High Five™ Cells with Plasmid DNA

Use this procedure to transfect Sf9, Sf21, or High Five™ insect cells in a 6-well format in Sf-900™ II SFM, Sf-900™ III SFM, Express™ Five SFM, or Grace's Insect Medium. All amounts and volumes are given on a per-well basis.

Timeline		Steps	Procedure Details
Day 1	1	Prepare plating medium and cells	At the time of transfection, ensure that you have $8 \times 10^5$ cells/well in a 6-well format in medium containing 1.5% FBS. <ul style="list-style-type: none"> <li>a. Prepare 10 mL of plating medium by mixing 1.5 mL Grace's Insect Medium, supplemented, containing 10% FBS (without antibiotics) and 8.5 mL Grace's Insect Medium, unsupplemented (without FBS or antibiotics).</li> <li>b. Plate <math>8 \times 10^5</math> of Sf9, Sf21, or High Five™ cells per well in 6-well plate. Allow the cells to attach for 15 minutes at room temperature in the hood. Remove medium and replace with 2.0 mL of plating medium.</li> </ul>
	2	Dilute Cellfectin™ II Reagent in Grace's Medium, Unsupplemented	<ul style="list-style-type: none"> <li>a. Mix the Cellfectin™ II Reagent before use.</li> <li>b. Dilute 8 µL in 100 µL Grace's Medium, Unsupplemented.</li> <li>c. Vortex briefly to mix.</li> </ul> <p><b>Note:</b> You may keep this mixture at room temperature for up to 30 minutes.</p>
	3	Dilute DNA in Grace's Medium, Unsupplemented	<p>Dilute 1–3 µg of plasmid DNA in 100 µL Grace's Insect Medium, Unsupplemented, and vortex briefly to mix.</p> <p>Optional: For High Five™ cells, add 5 µL of PLUS™ Reagent to diluted DNA, and incubate DNA:PLUS reagent for 5 minutes at room temperature.</p>
	4	Add diluted DNA to diluted Cellfectin™ II Reagent and incubate	Combine 100 µL of diluted DNA and 100 µL diluted Cellfectin™ II reagent, briefly vortex, and incubate at room temperature for 15–30 minutes.
Days 2–3	5	Add DNA-lipid complex to cells and incubate	Add DNA-lipid mixture drop-wise onto the cells. Incubate at 27°C for 3–5 hours.
	6	Replace media and incubate	Three to five hours post-transfection, replace media with 2 mL of either Sf-900™, Sf-900™ III, Express™ Five Medium, or Grace's Insect Medium containing 10% FBS with supplements. Incubate the cells at 27°C for 72 hours.