





 Package Contents	<table border="1"> <thead> <tr> <th>Catalog Number</th> <th>Amount</th> </tr> </thead> <tbody> <tr> <td>IVF3001</td> <td>10 reactions</td> </tr> <tr> <td>IVF3005</td> <td>50 reactions</td> </tr> </tbody> </table>	Catalog Number	Amount	IVF3001	10 reactions	IVF3005	50 reactions
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IVF3001	10 reactions						
IVF3005	50 reactions						
 Storage Conditions	<ul style="list-style-type: none"> Store at -20°C Invivofectamine® 3.0 Reagent can undergo a maximum of four freeze-thaw cycles without loss of activity. 						
 Required Materials	<ul style="list-style-type: none"> siRNA duplex (at 2.4 mg/mL in DNase/RNase-free water) Phosphate-Buffered Saline (PBS) pH 7.4 (Cat. no. 10010-23) DNase/RNase-Free Distilled Water (Cat. no. 10977) 						
 Timing	<p>Preparation: 10 minutes Incubation: 30 minutes</p>						
 Product Description	<p>Invivofectamine® 3.0 Reagent is an animal-origin-free lipid based <i>in vivo</i> RNAi transfection reagent designed for systemic siRNA delivery through tail vein injection.</p>						
 Important Guidelines	<ul style="list-style-type: none"> Use Ambion® <i>In Vivo</i> siRNA duplexes or Invitrogen <i>in vivo</i> Purity Stealth RNAi® siRNA duplexes formulated for animal use for <i>in vivo</i> RNAi experiments. Use a dose of 1 mg/kg as a starting point for experiments. This dose corresponds to 200 µL of a 0.1-mg/mL solution injected into a 20-g mouse (i.e. 10 µL/g). 						
 Online Resources	<p>Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.</p>						

Injection Techniques: General Concerns

Handle animals and conduct experiments according to national regulations and approvals by the local experimental ethics committee. All persons handling animals should be properly trained by the local facility. Weigh the animals prior to injection (200 µL standard injection volume; all calculations shown for a 20-g mouse, adjust volume accordingly for all other weights), and maintain a body-weight record over the course of the experiment. Use a mouse restraining device and/or a tail veiner (Braiintree Scientific) to ensure successful injection of your Invivofectamine® 3.0-siRNA solution.

Intravenous Injection

1. Restrain the mouse with a restraining device. Use a tail veiner to better visualize the tail.
2. For better visualization and dilation of the vein, warm the vein to ~37°C using a water bath or a heat lamp. Rotate the tail slightly to locate the vein.
3. Disinfect the injection site using an alcohol swab.
4. Insert the needle at a slight angle and inject slowly (20–40 µL/second) while watching for clearing of the blood. If resistance increases and a slight bulge appears in the tail, remove the needle and repeat the process proximal to the previous injection site.
5. After completing the injection, remove the needle and apply pressure to the injection site for ~5 seconds.

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


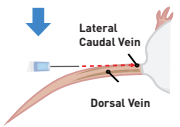
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Invivofectamine 3.0 Reagent Complexation Protocol

The following procedure outlines preparation of 1 mL of a 0.1-mg/mL complex in PBS starting with siRNA solutions having a final concentration of ≤ 0.1 mg/mL.

Steps		Procedure Details						
1	 <p>Complexation Buffer</p> <p>siRNA Duplex</p>	<p>Mix siRNA solution with Complexation Buffer</p> <table border="1"> <thead> <tr> <th>Component</th> <th>Volume</th> </tr> </thead> <tbody> <tr> <td>siRNA duplex solution (2.4-mg/mL)</td> <td>50 μL</td> </tr> <tr> <td>Complexation Buffer</td> <td>50 μL</td> </tr> </tbody> </table>	Component	Volume	siRNA duplex solution (2.4-mg/mL)	50 μ L	Complexation Buffer	50 μ L
Component	Volume							
siRNA duplex solution (2.4-mg/mL)	50 μ L							
Complexation Buffer	50 μ L							
2	 <p>Invivofectamine® 3.0 Reagent</p> <p>Diluted siRNA</p>	<p>Mix diluted siRNA with Invivofectamine 3.0 Reagent</p>						
3	 <p>Complex</p>	<p>Vortex</p>						
4	 <p>50°C</p> <p>30</p>	<p>Incubate</p>						
5	 <p>Complex</p>	<p>Dilute complex</p>						
6	 <p>Lateral Caudal Vein</p> <p>Dorsal Vein</p>	<p>Inject mouse</p>						

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