

# Lipofectamine® LTX & PLUS™ Reagent

## Protocol Outline

- Plate cells so they will be 70–90% confluent at the time of transfection.
- Prepare plasmid DNA-lipid complexes.
- Add DNA-lipid complexes to cells.

## Lipofectamine® LTX DNA Transfection Reagent Protocol

**i** See page 2 to view a typical plasmid transfection procedure.

## Transfection Amounts

Component	96-well	24-well	6-well
DNA per well	100 ng	500 ng	2500 ng
PLUS™ Reagent per well	0.1 µL	0.5 µL	2.5 µL
Lipofectamine® LTX Reagent per well	0.2–0.5 µL	1–2.5 µL	5–12.5 µL

## **i** Scaling Up or Down Transfections

## **i** Limited Product Warranty and Disclaimer Details

	<b>Package Contents</b>	<b>Catalog Number</b>	<b>Size: LTX/PLUS™</b>	
		▪ A12621	0.1 mL/40 µL	
		▪ 15338-030	0.3 mL/0.25 mL	*PLUS™ Reagent
		▪ 15338-100	1.0 mL/0.85 mL	(Cat. no. 11514-015)
		▪ 15338-500*	15 mL/-	available separately.

	<b>Storage Conditions</b>	▪ Store at 4°C (do not freeze).
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	<b>Required Materials</b>	▪ Plasmid DNA (0.5–5 µg/µL stock)
		▪ Opti-MEM® Reduced Serum Medium
		▪ Eppendorf tubes

	<b>Timing</b>	Preparation: 10 minutes
		Incubation: 5 minutes
		Final Incubation: 1-3 days

	<b>Selection Guide</b>	<a href="#">Lipofectamine® Reagents</a>
		Go online to view related products.

	<b>Product Description</b>	▪ Lipofectamine® LTX and PLUS™ Reagent are proprietary formulations for transfecting nucleic acids into a wide range of eukaryotic cells.
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	<b>Important Guidelines</b>	▪ DNA-Lipofectamine® LTX and PLUS™ complexes must be made in serum-free medium such as Opti-MEM® Reduced Serum Medium and can be added directly to cells in culture medium, in the presence or absence of serum/antibiotic.
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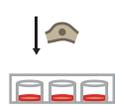
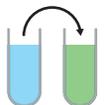
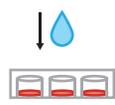
	<b>Important Guidelines</b>	▪ It is not necessary to remove complexes or change/add medium after transfection.
		▪ The amount of Lipofectamine® LTX Reagent required for successful transfection varies depending on the cell type and passage number. Start any new transfection by testing the recommended four concentrations of Lipofectamine® LTX Reagent to determine an optimum amount.

	<b>Online Resources</b>	Visit our <a href="#">product page</a> for additional information and protocols. For support, visit <a href="http://www.lifetechnologies.com/support">www.lifetechnologies.com/support</a> .
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## Lipofectamine® LTX DNA Transfection Reagents Protocol

Transfect cells according to the following table. Use the indicated volume of DNA and PLUS™ Reagent with each of the four volumes of Lipofectamine® LTX. **Each reaction mix is sufficient for triplicate (96-well), duplicate (24-well), and single well (6-well) transfections, and accounts for pipetting variations.** For even less toxicity, reduce the amount of DNA-lipid complex to the cells, or reduce the amount of DNA used to make complexes.

Timeline		Steps	Procedure Details				
Day 0	1	 Seed cells to be 70-90% confluent at transfection	Component	96-well	24-well	6-well	
	2	 Dilute Lipofectamine® LTX Reagent in Opti-MEM® Medium	Adherent cells	1–4 × 10 <sup>4</sup>	0.5–2 × 10 <sup>5</sup>	0.25–1 × 10 <sup>6</sup>	
Day 1	3	 Dilute DNA in Opti-MEM® Medium, then add PLUS™ Reagent	Opti-MEM® Medium	25 µL × 4	50 µL × 4	150 µL × 4	
	4	 Add diluted DNA to diluted Lipofectamine® LTX Reagent (1:1 ratio)	Lipofectamine® LTX Reagent	1, 1.5, 2, 2.5 µL	2, 3, 4, 5 µL	6, 9, 12, 15 µL	
	5	 Incubate	Opti-MEM® Medium	125 µL	250 µL	700 µL	
	6	 Add DNA-lipid complex to cells	DNA (0.5–5 µg/µL)	2.5 µg	5 µg	14 µg	
			PLUS™ Reagent	2.5 µL	5 µL	14 µL	
			Diluted DNA (with PLUS™ Reagent) Total	25 µL	50 µL	150 µL	
Day 2–4	7	 Visualize/analyze transfected cells	Diluted Lipofectamine® LTX Reagent	25 µL	50 µL	150 µL	
			Incubate for 5 minutes at room temperature.				
			Component	96-well	24-well	6-well	
			DNA-lipid complex per well	10 µL	50 µL	250 µL	
			Final DNA used per well	100 ng	500 ng	2500 ng	
			Final Lipofectamine® LTX Reagent used per well	0.2–0.5 µL	1.0–2.5 µL	5.0–12.5 µL	
			Incubate cells for 1–3 days at 37°C. Then, analyze transfected cells.				