

## Transfectamine™ 5000 Transfection Reagent

Catalog number: 60020, 60021, 60022

Unit size: 0.5 mL, 1 mL, 5 mL

Component	Storage	Amount		
		Cat No. 60020	Cat No. 60021	Cat No. 60022
Transfectamine™ 5000 Transfection Reagent	Freeze (<-15 °C), Minimize light exposure	0.5 mL	1 mL	5 mL

### OVERVIEW

Transfectamine™ 5000 Transfection Reagent is a powerful and versatile transfection reagent for the introduction of nucleic acids into eukaryotic cells, or more specifically, into animal cells. It can effectively transfect a variety of payloads into a variety of adherent and suspension cell lines. It can be used for plasmid DNA transfection as well as siRNA- and shRNA-based gene knockdown experiments and gene expression studies. It offers consistently high transfection efficiency in a wide variety of adherent and suspension cell lines, including difficult-to-transfect cells. The low toxicity of Transfectamine™ 5000 also allowed higher viability of transfected cells. Transfectamine™ 5000 is easier to use compare to most other transfection reagents and does not require special medium.

### AT A GLANCE

#### Protocol summary

1. Prepare cells for transfection
2. Prepare Transfectamine™ 5000-DNA mixture
3. Add Transfectamine™ 5000-DNA mixture to cell culture
4. Culture overnight
5. Analyze transfection efficiency with appropriate method

**Important** Thaw component at room temperature before starting the experiment.

### PREPARATION OF WORKING SOLUTION

1. Mix 2.5 ug of DNA with 200 uL of serum-free medium.
2. Add 7.5 uL of Transfectamine™ 5000 to Step 1.
3. Mix well and incubate at room temperature for 20 minutes.

**Note** Ratio of Transfectamine™ 5000 and DNA need to be optimized for different cell line, in general: Transfectamine™ 5000 Transfection Reagent (uL) to DNA (ug) Ratio = 3 - 5 uL to 1ug

#### Sample protocol detail for 6-well and 10 cm plate

Component	6 well plate (per well)	10 cm plate
Fresh culture medium	2 mL	6 mL
Plasmid	~2.5 ug	7.5~10 ug
Serum-free medium	200 uL	600 uL
Transfectamine™ 5000 Transfection Reagent	~7.5 uL	~22.5 uL

### SAMPLE EXPERIMENTAL PROTOCOL

#### Preparation of Cell Culture

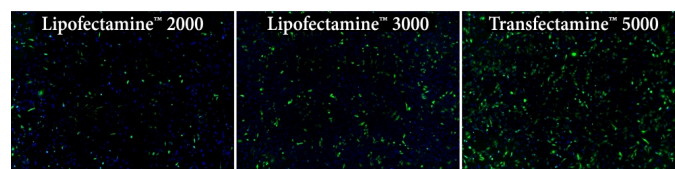
1. Culture cells to ~ 90% confluency at time of transfection.
2. Replace with fresh growth medium before transfection. For example, replace with 2 mL of medium per well for 6-well plates and 6 mL of medium for 10 cm plates.

#### Transection Protocol

1. Add Transfectamine™ 5000 -DNA mixture to culture plate and culture overnight.

**Note** Recombinant protein can start to be detected as early as 16 hours post transfection. Maximal expression level may be observed 72~96 hours post transfection.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Transfection efficiency comparison in HeLa cells using Transfectamine™ 5000, Lipofectamine 2000 and Lipofectamine 3000 reagents. Each reagent was used to transfect HeLa cells in a 96-well format, and GFP expression was analyzed 24 hours post-transfection. Transfectamine™ 5000 transfection reagent provided higher GFP transfection efficiency compared to Lipofectamine 2000 and Lipofectamine 3000 reagents.

### DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.