

# BioT Transfection Reagent

(Lipid based, low toxicity)

Catalog # BT0100

1 mL



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## Plasmid DNA Transfection Protocol:

The following procedure is used (as an example) to transfect DNA into mammalian cells in a 35 mm dish or 6 well plate. Refer to Table 1 to scale up or down the protocol when using other sized dishes or plates. All amounts and volumes are given on a per well basis. Prepare complexes using a ratio of DNA ( $\mu\text{g}$ ) : BioT ( $\mu\text{L}$ ) = 1 : 1.5 for most cell lines or primary cells. Optimization may be required (see Appendices A & B, page 3).

1. One day before transfection, split cells so that they will reach 60-75% confluence on the day of transfection.
2. On the day of transfection, change the cell culture medium (plating medium), adding 2 mL. Prepare the transfection complexes for each sample as described below. In a sterile 1.5 mL microcentrifuge tube, add and mix the following reagents sequentially:

100 $\mu\text{L}$	Mixing Medium: Serum-free DMEM (or any other serum-free medium such as PBS) <b>Please do not use OPTI-MEM</b>
2 $\mu\text{g}$	Plasmid DNA
3 $\mu\text{L}$	BioT

3. Pipette the transfection complex a few times to mix, spin briefly in a centrifuge and incubate the complex at room temperature for 5 minutes.
4. Add the entire complex directly to the plated cells. Tilt back and forth the dish or plate a few times to mix the complex into the medium and return it to the CO<sub>2</sub> incubator. Serum concentration in the growth medium has no effect on transfection efficiency. As much as 20% fetal bovine serum has been tested without significantly changing transfection efficiency.
5. 16 to 24 hours after transfection, change the culture medium. If no toxicity is observed, change of medium is not required. If high toxicity is observed, either reduce the amount of DNA and/or shorten incubation time to 5-8 hours.
6. Peak protein expression following transfection using BioT is seen at 36-48 hours post-transfection.

**Table 1. Scaling up or down plasmid DNA transfections (based on plating medium volume):**

Cell Culture Dish/Plate	Surface Area/Well	Volume of Plating Medium	Volume of Mixing Medium	Plasmid DNA	BioT
10 cm	56 cm <sup>2</sup>	10 mL	500 $\mu\text{L}$	10 $\mu\text{g}$	15 $\mu\text{L}$
60 mm	21 cm <sup>2</sup>	3 mL	150 $\mu\text{L}$	3 $\mu\text{g}$	4.5 $\mu\text{L}$
35 mm	8 cm <sup>2</sup>	2 mL	100 $\mu\text{L}$	2 $\mu\text{g}$	3 $\mu\text{L}$
6 well	9.5 cm <sup>2</sup>	2 mL	100 $\mu\text{L}$	2 $\mu\text{g}$	3 $\mu\text{L}$
12 well	3.8 cm <sup>2</sup>	1 mL	50 $\mu\text{L}$	1 $\mu\text{g}$	1.5 $\mu\text{L}$
24 well	1.9 cm <sup>2</sup>	0.5 mL	25 $\mu\text{L}$	0.5 $\mu\text{g}$	0.75 $\mu\text{L}$
48 well	0.95 cm <sup>2</sup>	0.25 mL	12.5 $\mu\text{L}$	0.25 $\mu\text{g}$	0.37 $\mu\text{L}$
96 well	0.32 cm <sup>2</sup>	0.1 mL	5 $\mu\text{L}$	0.1 $\mu\text{g}$	0.15 $\mu\text{L}$

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### siRNA Transfection Protocol:

The following procedure is used (as an example) to transfect siRNA into mammalian cells in a 12 well plate. Refer to Table 2 to scale up or down the protocol when using other sized dishes or plates. All amounts and volumes are given on a per well basis. Optimization may be required (see Appendix D, page 4).

1. One day before transfection, split cells so that they will reach 50-70% confluence on the day of transfection.
2. On the day of transfection, change the cell culture medium (plating medium), adding 1 mL. Prepare the transfection complexes for each sample as described below. In a sterile 1.5 mL microcentrifuge tube, add and mix the following reagents sequentially:

50 $\mu$ L	Mixing Medium: Serum-free DMEM (or any other serum-free medium such as PBS) <b>Please do not use OPTI-MEM</b>
40 pmol	siRNA (2 $\mu$ L x 20 $\mu$ M siRNA)
1 $\mu$ L	BioT

3. Pipette the transfection complex a few times to mix, spin briefly in a centrifuge and incubate the complex at room temperature for 5 minutes.
4. Add the entire complex directly to the plated cells. Tilt back and forth the dish or plate a few times to mix the complex into the medium and return it to the CO<sub>2</sub> incubator. Serum concentration in the growth medium has no effect on transfection efficiency. As much as 20% fetal bovine serum has been tested without significantly changing transfection efficiency.
5. 16 to 24 hours after transfection, change the culture medium. If no toxicity is observed, change of medium is not required. If high toxicity is observed, either reduce the amount of DNA and/or shorten incubation time to 5-8 hours.
6. Gene knockdown following transfection using BioT should be assayed 24-72 hours post-transfection.

**Table 2. Scaling up or down siRNA transfections (based on plating medium volume):**

Cell Culture Dish/Plate	Surface Area/well	Volume of Plating Medium	Volume of Mixing Medium	siRNA*	BioT
10 cm	56 cm <sup>2</sup>	10 mL	500 $\mu$ L	400 pmol (100-500 pmol)	10 $\mu$ L
60 mm	21 cm <sup>2</sup>	3 mL	150 $\mu$ L	120 pmol (30-150 pmol)	3 $\mu$ L
35 mm	8 cm <sup>2</sup>	2 mL	100 $\mu$ L	80 pmol (20-100 pmol)	2 $\mu$ L
6 well	9.5 cm <sup>2</sup>	2 mL	100 $\mu$ L	80 pmol (20-100 pmol)	2 $\mu$ L
12 well	3.8 cm <sup>2</sup>	1 mL	50 $\mu$ L	40 pmol (10-50 pmol)	1 $\mu$ L
24 well	1.9 cm <sup>2</sup>	0.5 mL	25 $\mu$ L	20 pmol (5-25 pmol)	0.5 $\mu$ L
48 well	0.95 cm <sup>2</sup>	0.25 mL	12.5 $\mu$ L	10 pmol (2.5-12.5 pmol)	0.25 $\mu$ L
96 well	0.32 cm <sup>2</sup>	0.1 mL	5 $\mu$ L	4 pmol (1-5 pmol)	0.1 $\mu$ L

\*20  $\mu$ M siRNA = 20 pmol/ $\mu$ L

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### Appendix A: Optimization of single plasmid transfections:

For untested cell lines, use the following table to optimize transfection complex ratios:

DNA	0.5 µg	1.0 µg	2.0 µg	3.0 µg	4.0 µg
BioT	1.0 µL	1.5 µL	3.0 µL	4.5 µL	6.0 µL

Use the following characteristics and table to optimize transfection efficiency:

- ▶ Increasing the amount of DNA and keeping the DNA : BioT ratio the same will increase transfection efficiency but may increase toxicity
- ▶ Decreasing the amount of DNA and keeping the DNA : BioT ratio the same will decrease transfection efficiency and toxicity
- ▶ Keeping the amount of DNA the same and decreasing the DNA : BioT ratio to 1 : 1 will decrease the toxicity but may decrease transfection efficiency

Condition	DNA	DNA : BioT
Cellular Toxicity	0.5-1 µg	1 : 1.5-2.0
	2 µg	1 : 0.5-1.0
Low Transfection Efficiency	3 µg	1 : 1.5-2.0
	4 µg (for very difficult-to-transfect cells)	1 : 1.5-2.0
	5-6 µg (for extremely difficult-to-transfect cells)	1 : 1.5-2.0

### Appendix B: Optimization of multiple plasmid transfections:

Because of BioT's low cost and high transfection efficiency in 293, 293T and Phoenix cells, using BioT to make retroviruses and lentiviruses is an ideal choice. Currently there are numerous vectors used to make retro- and lentiviruses.

The ratio of vector DNA : BioT should be kept the same as suggested when transfecting single plasmids and siRNA (1 : 1.5). The total amount of vector DNA required for successful transfection is as little as one-half to two-thirds the amount of DNA required to produce viruses using Lipofectamine™ 2000 (Life Technologies, Carlsbad, CA).

The volume of mixing medium should be adjusted accordingly. Mixing Medium : DNA = 50 µL : 1µg. The tables below provides optimization examples. Medium (containing viruses) can be collected at 48, 72 and 96 hours post-transfection. Some labs also collect viruses at 12 hour intervals from 36 to 96 hours post-transfection. Assay viral titers at each time point.

3-Vector Lentivirus System (Medium : DNA : BioT = 50: 1 : 1-1.5)			
Plasmid	Amounts & Volumes of DNA & Medium (per 10 cm Cell Culture Dish)		
PMDG1 (envelope, VSV-G)	2.5 µg	2 µg	1.5 µg
PCMV DR8.91 (packaging, gag-pol-rev)	7.5 µg	6 µg	4.5 µg
Lenti-vector (transfer vector)	10.0 µg	8 µg	6.0 µg
Total amount of DNA	20 µg	16 µg	12 µg
Mixing medium	1 mL	800 µL	600 µL
BioT	30 µL	24 µL	18 µL

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### Appendix B continued:

4-Vector Lenti-virus System (Medium : DNA : BioT = 50: 1 : 1-1.5)	
Plasmid	Amounts & Volumes of DNA & Medium (per 10 cm Cell Culture Dish)
rev (RSV/rev)	3 µg
gp (MDL/gag-pol)	5 µg
VSV-G (CMV/VSV-G)	1.5 µg
Transfer vector (HIV-7)	10.5 µg
Total amount of DNA	20 µg
Mixing medium	1 mL
BioT	30 µL

### Appendix C: Cell-specific dosages of BioT:

The following tables lists amounts of BioT used in some specific cell types (per 35 mm dish or 6 well plate)

Cell Type	Plasmid	BioT
C2C12	4 µg	6 µL
	6 µg	10 µL
MCF-7	3 µg	4.5 µL
MCF10A	0.5 µg	1 µL
	1 µg	1 µL
	1 µg	1.5 µL
Mouse mesenchymal stem cells	1 µg	1.5 µL
	1 µg	1 µL

### Appendix D: Optimization of siRNA transfections:

Although siRNA transfections are optimal when present in cell culture medium at 10-50 nM concentration, consider analyzing a broader range of concentrations (e.g. 1-100 nM). For each new siRNA, optimization is required to obtain the highest transfection efficiency or best gene knockdown result.

Please feel free to contact us with any technical questions:

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