BioT

Cell Transfection Manual

For Research Use Only. Not For Application To Humans.

(October 2020)

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BioT

(Catalog#: B01-01, 1ml; B01-02, 4x1ml)

An exceptionally efficient and non-toxic transfection reagent for a broad range of mammalian and insect cell types

BioT is a lipid-based reagent designed and formulated with proprietary technology for transfecting DNA, siRNA and anti-sense oligonucleotides into a variety of eukaryotic cell lines, insect cells, and cells of primary culture and suspension culture. **BioT** offers extremely high transfection efficiencies and minimal cytotoxicity. The reagent works very well in cultures with fetal bovine serum (FBS) concentration range from 0 to 20%. One milliliter (1.0 ml) of **BioT** is sufficient for more than 300 transfections in 6-well plates or 35 mm dishes.

Major Features

- Exceptional efficiency of transfection for a broad range of adherent cell types.
- Especially good for embryonic stem (ES) cells and difficult-to-transect primary cultures.
- Equally efficient for suspension cultures of HEK293, and sf9, sf21 and High5 insect cells.
- Higher efficiency and lower toxicity than all commercially available transfection reagents currently on the market.
- Transfection efficiency not affected by serum. Works equally well in serum-containing (up to 20% FBS) or serum-free media.
- Non-toxic or minimal cytotoxicity.
- Extremely simple protocol for transfection, using only one reagent without the need for diluents or enhancers.
- High levels of recombinant protein production at 24-72 hours after transfection.

- Works very well for both single DNA transfection and multi-DNA co-transfection.
- Low cost (Almost 50% price off compare to other major commercial products).

Storage Condition

BioT is stable at room temperature. It is recommended to be stored sterile at 4 °C. It is stable for 18 months or longer.

Important Note

1. BioT is a proprietary product of Bioland Scientific LLC and its partner, any material transfer without permission is prohibited.
2. BioT reagent is designed for *in vitro* use only. It should never be applied to animals or human.

Selected publications:

1. Control of iron homeostasis by an iron-regulated ubiquitin ligase. Vashisht AA, Zumbrennen KB, Huang X, Powers DN, Durazo A, Sun D, Bhaskaran N, Persson A, Uhlen M, Sangfelt O, Spruck C, Leibold EA, Wohlschlegel JA. **Science.** 2009 Oct 30; 326 (5953): 718-21.
2. Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. Bloom JD, Gong LI, Baltimore D. **Science**. 2010 Jun 4;328(5983):1272-5.
3. Antibody-based protection against HIV infection by vectored immunoprophylaxis.Balazs AB, Chen J, Hong CM, Rao DS, Yang L, Baltimore D. **Nature.** 2011 Nov 30;481(7379):81-4.
4. Regulation of an RNA granule during spermatogenesis: acetylation of MVH in the chromatoid body of germ cells.Nagamori I, Cruickshank VA, Sassone-Corsi P. **J Cell Sci.** 2011 Dec 15;124(Pt 24):4346-55.
5. Regulation of Adipose Differentiation by Fructose and GluT5. Du L, Heaney AP. **Mol Endocrinol.** 2012 Jul 24.
6. MMS19 assembles iron-sulfur proteins required for DNA metabolism and genomic integrity. Stehling O, Vashisht AA, Mascarenhas J, Jonsson ZO, Sharma T, Netz DJ, Pierik AJ, Wohlschlegel JA, Lill R. **Science.** 2012 Jul 13;337(6091):195-9.
7. Serine phosphorylation by SYK is critical for nuclear localization and transcription factor function of Ikaros. Uckun FM, Ma H, Zhang J, Ozer Z, Dovat S, Mao C, Ishkhanian R, Goodman P, Qazi S. **Proc Natl Acad Sci USA.** 2012 Oct 30;109(44):18072-7.

Plasmid DNA Transfection Protocol

The following procedure transfects DNA into mammalian cells in a 35 mm dish. For other size of cell culture dish, refer to Table 1 for scaling up and down. All amounts and volumes are given on a per well basis. Prepare complexes using a ratio of **DNA** (µg)**: BioT** (µl) **= 1:1.5** (Range of 1:0.5-2) for most cell lines or primary cells. Optimization may be necessary (see Page 5).

1. **Split the cells one day before transfection to reach 60 – 75% confluence in the day of transfection.**
2. **For 35 mm culture dish, change the growth medium to 2 ml in the day of transfection.**
3. **For each transfection sample, prepare the transfection complexes as below:** In a sterile 1.5 ml microtube, mix the following reagents:

100 µl	**Serum-free DMEM (or any other serum-free medium, PBS, DPBS etc.). Do not use OPTI-MEM!**
2 µg	**Plasmid DNA**
3 µl	**BioT**

4. **Pipetting up and down the mixture a few times. Spin briefly in a centrifuge. Leave the mixture at room temperature for 5 min.**
5. **Add the entire mixture directly to cells in the 35 mm culture dish. Tilting the dish a few times to mix. Return the dish to a CO_2 incubator.** Serum concentration in the growth medium has no effect on the transfection efficiency. Up to 20% of fetal bovine serum has been tested without significantly changing the transfection efficiency.
6. **16 to 24 hours after transfection, replace the medium in the dish with 2 ml (or any volume appropriate) fresh growth medium.** Note: If no toxicity is observed, change of medium is not needed. If high toxicity is observed, either adjusts the amount of DNA and/or shortens incubation time to 5-8 hours.

7. **Protein expression should reach a high level at or after 48 hours post-transfection.**

Table1. Scaling Up or Down Transfections (based on plating medium volume)

Culture dish	Surf. Area per well (cm^2)	Shared reagents		DNA transfection	
		Vol. Of Plating Medium	Vol. Of mixing Medium	DNA	BioT
10 cm	56	10 ml	500 µl	10 µg	15 µl
60 mm	21	3 ml	150 µl	3 µg	4.5 µl
35 mm	8	2 ml	100 µl	2 µg	3 µl
6-well	9.5	2 ml	100 µl	2 µg	3 µl
12-well	3.8	1 ml	50 µl	1 µg	1.5 µl
24-well	1.9	0.5 ml	25 µl	0.5 µg	0.75 µl
48-well	0.95	0.25 ml	12.5 µl	0.25 µg	0.37 µl
96-well	0.32	0.1 ml	5 µl	0.1 µg	0.15 µl

Optimization Procedure

1. **For new cell lines, use the table below to choose the condition**

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

2. **Use the following table to fine-tune the transfection**

- **Increase DNA amount, keep DNA:BioT ratio the same, will increase the transfection efficiency and may also increase toxicity.**
- **Decrease DNA amount, keep DNA:BioT ratio the same, will decrease the toxicity and efficiency.**
- **Keep DNA amount the same, decrease DNA:BioT ratio (to 1:0.5-1), will decrease the toxicity and may also decrease the efficiency.**

In case of	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

siRNA Transfection Protocol

The procedure in the example is used to transfect siRNA into mammalian cells in a 12-well dish. For other size of cell culture dish, refer to Table 1 for scaling up and down. All amounts and volumes are given on a per well basis. Optimization is necessary for any new siRNA (see Page 8).

1. **Split the cells one day before transfection to reach 70 – 80% confluence in the day of transfection.**
2. **In the day of transfection, prepare the transfection complexes as below:** In a sterile 1.5 ml microtube, mix the following reagents (for 12-well dish):

50 µl	Serum-free DMEM (or any other serum-free medium, PBS, DPBS etc.). **Please do not use OPTI-MEM!**
40 pmol	siRNA (2 µl x 20 µM siRNA)
1 µl	BioT

3. **Pipetting up and down the mixture a few times. Spin briefly in a centrifuge. Leave the mixture at room temperature for 5 min.**
4. **Add the entire mixture directly to the medium of the 12-well culture dish. Tilting the dish a few times to mix. Return the dish to a CO_2 incubator.** Serum concentration (0-20%) in the growth medium has no effect on the transfection efficiency.
5. **16 to 24 hours after transfection, replace the medium in the dish with 2 ml (or any volume appropriate) fresh growth medium.** Note: If no toxicity is observed, change of medium is not needed. If high toxicity is observed, either adjusts the amount of siRNA and/or shortens incubation time to 5-8 hours.
6. **Gene knockdown should be assayed 48-72 hours post-transfection.**

Table 1. Scaling Up or Down Transfections (Based on plating-medium volume, 20 µM siRNA=20 pmol/µ

Culture dish	Surf. Area per well (cm^2)	Shared reagents		RNAi transfection	
		Vol. Of Plating Medium	Vol. Of mixing Medium	siRNA* Range	BioT
10 cm	56	10 ml	500 µl	400 pmol (100-500 pmol)	10 µl
60 mm	21	3 ml	150 µl	120 pmol (30-150 pmol)	3 µl
35 mm	8	2 ml	100 µl	80 pmol (20-100 pmol)	2 µl
6-well	9.5	2 ml	100 µl	80 pmol (20-100 pmol)	2 µl
12-well	3.8	1 ml	50 µl	40 pmol (10-50 pmol)	1 µl
24-well	1.9	0.5 ml	25 µl	20 pmol (5-25 pmol)	0.5 µl
48-well	0.95	0.25 ml	12.5 µl	10 pmol (2.5-12.5 pmol)	0.25 µl
96-well	0.32	0.1 ml	5 µl	4 pmol (1-5 pmol)	0.1 µl

Optimization Procedure for siRNA transfection

siRNAs typically work best when present in cell culture medium at 10-50 nM. But a more extensive concentration range from 1-100 nM can be analyzed in optimization experiments. For each new siRNA, optimization is required to obtain the highest transfection efficiency or best gene knockdown result. For example: using 12-well dish, keep the amount of BioT the same (1 µl), varying the siRNA amount at 5, 10, 20, 40 and 100 pmol. Assay the gene knockdown 48-72 hours post-transfection

Optimization Procedure for siRNA transfection

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III: Plasmid DNA and siRNA Co-transfection Protocol

Follow the same procedure as siRNA transfection. Refer to the following table for the reagent amounts and volumes. Cells should be harvested 24-72 hours post-transfection. (20 µM siRNA=20 pmol/µl)

Culture dish	Shared reagents		Co-transfection		
	Vol. Of Plating Medium	Vol. Of mixing Medium	Plasmid DNA	siRNA Suggested (Range)	BioT Suggested (Range)
10 cm	10 ml	500 µl	3 µg	100 pmol (20-250)	10 µl (5-15)
60 mm	3 ml	150 µl	900 ng	30 pmol (5-75)	3 µl (1.5-4.5)
35 mm	2 ml	100 µl	600 ng	20 pmol (5-50)	2 µl (1-3)
6-well	2 ml	100 µl	600 ng	20 pmol (5-50)	2 µl (1-3)
12-well	1 ml	50 µl	300 ng	10 pmol (2-25)	1 µl (0.5-1.5)
24-well	500 µl	25 µl	150 ng	5 pmol (1-12.5)	0.5 µl (0.25-0.75)
48-well	250 µl	12.5 µl	75 ng	2.5 pmol (0.5-6.25)	0.25 µl (0.12-0.4)
96-well	100 µl	5 µl	30 ng	1 pmol (0.1-2)	0.1 µl (0.05-0.15)

Optimization Procedure for plasmid - siRNA co-transfection

For each new co-transfection, optimization is required to obtain the best gene knockdown result: using 12-well dish, keep the amounts of BioT and plasmid the same (1 µl and 300 ng respectively), varying the siRNA amount at 2, 5, 10, and 25 pmol. To obtain highest plasmid transfection efficiency: keep the amounts of BioT and siRNA the same, varying the plasmid amount at 200, 300 and 400 ng. Assay the gene knockdown and plasmid expression 48-72 hours post-transfection.

Appendix A: List of cells using BioT for transfection

Cell lines	ST-2
HEK293	10T1/2
CHO	T47D
COS	HepG2
NIH3T3	TC-32
HeLa	**Primary Culture**
C2C12	Mouse embryonic stem cells
MCF-7	Mouse embryonic fibroblasts
MCF10A	Mouse mesenchymal stem cells
MNTT-1	Mouse astrocytes
NCCIT	Mouse cortical neurons
PC12	Rat cortical neurons
RAW264.7	Rat adrenal chromaffin cells
RKO	Hepatocytes
ROS17-2	**Suspension Culture**
S2 (Schneider) Drosophial cells	HEK 293
SaOS2	Insect cells (sf9, sf21, High-Five)

Appendix B: Cell specific dosage of BioT

The amount of BioT using in some specific cells are listed in the following table (based on 35 mm dish or 6-well plate).

Cells	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	1.5 μl
Mouse mesenchymal stem cells	1 μg	1.5 μl

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