

EzTransfactor™ Transfection Reagent

For the transient and stable transfection of animal cells

| | |
|---------------------------|------------------------------------------|
| Cat. No. R0701-05 | 0.5 ml (up to 250 transfections) |
| Cat. No. R0701-10 | 1 ml (up to 500 transfections) |
| Cat. No. R0701-20 | 2 ml (up to 1000 transfections) |
| Cat. No. R0701-100 | Multi-pack 5 × 2 ml, individually packed |

Version October 2009

Store at +2 to +8°C

Made in U.S.A.

To order bulk package, please call 1-866-897-4664

Principle Description

Eztransfactor™ Transfection Reagent is a cationic polymer transfection reagent. This cationic polymer conjugates peptides which binding cell surface receptors. The solution is stable at 4°C. Stability decreases when buffered. The efficient gene transfer activity of Eztransfactor™ is related to its capacity for condensing DNA and bind to the cell surface. Eztransfactor™ has a high cationic-charge density potential which makes it an excellent DNA condensing and gene-delivery agent. Eztransfactor™ provides superior transfection efficiency and significantly lower toxicity when compared to other cationic lipids and polymers in a wide number of cell types.

Eztransfactor™ is suitable for transfection with high efficiency and minimal toxicity. **Transfection can be performed in the presence or absence of serum** (Eztransfactor™ efficiency is not affected by the presence of serum). Eztransfactor™ showed superior results when compared to many other lipid or polymer based transfection reagents.

What this Product Does

Number of Transfection Experiments

Using standard experimental conditions, Eztransfactor™ Transfection Reagent can be used to perform up to five hundred transfections in 35-mm tissue-culture dishes that contain HeLa, A293, or BAEC cells. This is equivalent to over 2,000 transfections in 24-well plates.

Storage and Stability

Store Eztransfactor™ Transfection Reagent at +2 to +8°C, with a tightly closed lid. The reagent is stable through the expiration date printed on the label when stored under these conditions. The solution is stable at 4°C for 12 months, and stability decreases when buffered. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Further dilutions can be made in assay buffer.

How to Use this Product

Read the information below carefully before you begin:

- DNA quality:** High quality DNA is critical for successful transfection. OD260/OD280 ratio of 1.8 or greater is recommended. DNA should be sterile and free of any contaminant such as endotoxins.
- Cell density at transfection:** The recommended cell density (confluence) for most cell types is 50-70% at the time of the transfection. The cells should not be confluent or at a stationary phase at the time of transfection.
- Transfection incubation time:** Detection of transient expression of genes takes place within 6-48 hours. The optimal post-transfection incubation time can be determined and monitored using a reporter gene such as Luciferase, -galactosidase or Green Fluorescent Protein
- Eztransfactor™/DNA ratio: From 1:1 to5: 1** or the required amount of Eztransfactor™ depends on the amount of DNA and the number of equivalents needed. A gradient ratio experiment for the first time user is strongly recommended.

Ratio Overview:

Preparation of a transfection complex that is sufficient for a 35-mm culture dish, or one well of a six-well plate, at six different ratios

| Tube Label | Medium (μl) | Eztransfactor™ (μl) | DNA (μg) | Comments |
|------------|-------------|---------------------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1:1 | 99 | 1 | 1 | Add the entire volume to each well of a six-well plate, or 5 μl to each well of a 96-well plate. Additional suggested volumes for different containers are included in Table 1. |
| 2:1 | 98 | 2 | 1 | |
| 5:2 | 95 | 5 | 2 | |

- Transfection in the presence of serum and antibiotics:** Eztransfactor-mediated transfection efficiency is not affected by the presence of serum and antibiotics. In fact, the transfection efficiency is higher in the presence of serum due to the better survival rate of cells. Replace full medium by antibiotics free medium if you observe more than 5-10% cell death.

6. **High Protein-Expression Levels:** High expression levels of certain intracellular proteins (e.g., GFP) may be cytotoxic to some cell types.

Transfection Procedure

Easy and fast. Not affected by the presence of serum or antibiotics. No medium change required.

1. Seed 5×10^5 cells per well (6-well plate) the day before transfection.
2. Label three sterile tubes: "2:1", "5:2" and "5:1". Pipet 98 μ l of medium into the first tube and 95 μ l into the last two tubes. Then pipet the Eztransfactor™ transfection reagent directly into the medium: 2 μ l reagent into the first tube and 5 μ l into

each of the last two tubes. Vortex for one second or flick the tube to mix.

3. Add 1 μ g of plasmid DNA into each of the tubes labeled "2:1" and "5:1" and 2 μ g DNA into the tube labeled "5:2." Limit the total volume of added DNA solution to between 0.5 μ l and 50 μ l. Vortex immediately and incubate the complex for 30 min at room temperature.
4. Add the transfection reagent/ DNA complex to the cells. Swirl the wells or flasks to ensure equal distribution over the entire plate surface. Incubate cells for 6-48 hours.

Table 1.

Eztransfactor™ Reagent: DNA Complex preparation for Various Culture Vessel Sizes*

| Container | Surface area (cm ²) | Typical total volume of medium (ml) | Starting volume of Eztransfactor™ (μl) | | Starting mass of DNA (μg) | | Approximate total volume of complex (μl) |
|---------------|---------------------------------|-------------------------------------|----------------------------------------|-----------|---------------------------|-----------|------------------------------------------|
| | | | μl Amount | μl Range | μg Amount | μg Range | |
| 96 well | 0.3 | 0.1-0.2 | 0.1 | 0.05-0.25 | 0.05 | 0.03-0.06 | 5 |
| 24 well | 1.9 | 0.5-2.0 | 0.4 | 0.2-1.0 | 0.2 | 0.2-0.4 | 20 |
| 12 well | 3.8 | 0.5-1.0 | 1.0 | 0.5-2.5 | 0.5 | 0.4-0.8 | 50 |
| 6 well (35mm) | 8 | 2.0 | 2.0 | 1.0-10.0 | 1.0 | 1.0-2.0 | 100 |
| 60 mm | 21 | 4.0-6.0 | 4.0 | 2.0-10.0 | 2.0 | 2.0-4.5 | 200 |
| 10 cm | 55 | 10.0 | 12.0 | 6.0-30.0 | 6.0 | 5.6-11.0 | 600 |
| T-25 | 25 | 5.0-7.0 | 5.0 | 2.5-12.5 | 2.5 | 2.5-5.3 | 250 |
| T-75 | 75 | 15.0-30.0 | 16.0 | 8.0-40.0 | 8.0 | 8.0-16.0 | 800 |

*Refer to the table below when setting up your transfection reactions. The starting volume and mass is based on a Eztransfactor™ Transfection Reagent : DNA ratio of 2:1. The ranges cover ratios of 1:1, 2:1, and 5:1. When using the upper range of the DNA mass, use only the upper range of the Eztransfactor™ Transfection Reagent volumes. When using the lower range of the DNA mass, the entire range of Eztransfactor™ Transfection Reagent volume may be tested.

Quality Control

Transfection efficiency was tested on BAEC cells, with 1 μ g of EYFP plasmid DNA and oligo nucleotide by using Eztransfactor™ Transfection Reagent in 6-well plate. Transfection efficiency, i.e. the percentage of transfected cells, is $90.0 \pm 5\%$.

Order information

TSZGENE Eztransfactor™ Transfection Reagent is manufactured by TSZ Scientific LLC company and distributed by Biotang Inc. in USA. For a complete overview of related products and manuals please visit and bookmark our website www.tszgene.com and our distributor's homepage, www.biotangusa.com
 BIOTANG Inc.
 27 Pembroke st.
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Important Note

This product as supplied is intended for research use only, NOT for use in human, therapeutic or diagnostic applications without the expressed written authorization of United States Biological.

Purchaser represents and warrants that it will use Eztransfactor™ Transfection Reagent purely for research purposes. Transfected cells, materials produced, and any data derived from the use of Eztransfactor™ Transfection Reagent, may be used only for the internal research of Purchaser whether Purchaser is a "for-profit" or a "not-for-profit" organization. Under no circumstances may Eztransfactor™ Transfection Reagent be used by Purchaser or any third party for a commercial purpose unless Purchaser has negotiated a license for commercial use with TSZ Scientific LLC. (Contact [information: contact@tszgene.com](mailto:contact@tszgene.com)). For purposes of the foregoing sentence, "commercial purpose" shall mean use of Eztransfactor™ Transfection Reagent for profit or commercial gain. By using Eztransfactor™ Transfection Reagent, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the Eztransfactor™ Transfection Reagent to TSZ Scientific LLC. for a full refund.