

## Altogen Biosystems

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### **bEnd-3 Transfection Protocol (Kit for Brain Endothelioma Cells, CRL-2299)**

**Catalog No. 6145**    Size: 0.5 ml

**Catalog No. 6146**    Size: 1.5 ml

**Catalog No. 6147**    Size: 8.0 ml

#### **Contents and Shipping:**

bEnd-3 Transfection Kit includes bEnd-3 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). bEnd-3 Transfection Reagent is supplied in liquid form at a concentration of 0.35 mg/ml, shipped at ambient temperature.

#### **Description:**

Nanoparticle-based liposome transfection reagent is a proprietary formulation optimized for transfection of DNA and RNA into bEnd-3 cells.

#### **Product Qualification:**

bEnd-3 Transfection Reagent is tested functionally by transfection of bEnd-3 cells with a small interfering RNAs targeting 3 different genes (Lamin A/C, GAPDH, Cyclophilin B). Transfection Reagent is tested for absence of nuclease contamination and microbial contamination.

#### **Storage:**

Store reagent at 4°C upon receipt. If stored properly, reagent is stable for 12 months.

#### **Intended Use:**

For *in vitro* use only.

#### **Safety Data Sheet (SDS):**

SDS documents are available online at <https://altogen.com>

#### ***In Vivo* Transfection Kits (for compound testing in rodents):**

- Catalog #5010 / 5011 / 5012 - Lipid *In Vivo* Transfection Kit
- Catalog #5020 / 5021 / 5022 - Polymer *In Vivo* Transfection Kit
- Catalog #5030 / 5031 / 5032 - Nanoparticle *In Vivo* Transfection Kit
- Catalog #5040 / 5041 / 5042 - PEG-Liposome *In Vivo* Transfection Kit
- Catalog #5050 / 5051 / 5052 - Pancreas *In Vivo* Transfection Kit
- Catalog #5060 / 5061 / 5062 - Liver *In Vivo* Transfection Kit
- Catalog #5070 / 5071 / 5072 - Kidney *In Vivo* Transfection Kit

#### **Transfection Controls and Recommended Products:**

- Catalog #4060 - GFP-expressing plasmid DNA (25 ug)
- Catalog #4061 - Cell Cycle Arrest siRNA (5 nmol)
- Catalog #4062 - Apoptosis Inducing siRNA (5 nmol)

#### **To Place an Order:**

Both domestic (USA) and international orders can be placed online (<https://altogen.com>) using credit card payment. Purchase Order (PO) can be faxed at (702) 989-0841 (for USA only).

**Transfection Resource:**    [altogen.com/transfection-resource](http://altogen.com/transfection-resource)

**Altogen Labs. GLP Compliant Pre-clinical CRO Laboratory Services:**

[AltogenLabs.com](http://AltogenLabs.com)

## Recommended Transfection Protocols (for 24-well plate):

<b>bEnd-3 Standard Transfection Protocol (24-well plate):</b>	<b>bEnd-3 Reverse Transfection Protocol (24-well plate):</b>
<ol style="list-style-type: none"> <li>1. Plate 15,000 - 20,000 bEnd-3 cells per well in 0.5 ml of complete growth medium 12–24 hours prior to transfection</li> <li>2. Wash with 1xPBS and add 0.5 ml of fresh growth medium</li> <li>3. Prepare transfection complexes by mixing 40 µl of serum-free medium, 5.5 µl of transfection reagent, and <ul style="list-style-type: none"> <li>• 750 ng DNA (or mRNA), or</li> <li>• 30 nM - 50 nM of siRNA (or microRNA)</li> </ul> <i>*Referred to a final volume including growth medium</i> </li> <li>4. Incubate transfection complexes at RT for 15 - 30 minutes</li> <li>5. <u>Optional:</u> Add 2 µl of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity</li> <li>6. Add prepared transfection complexes to 0.5 ml of complete growth medium with bEnd-3 cells (from step 2)</li> <li>7. Incubate cells at 37°C in a humidified CO<sub>2</sub> incubator</li> <li>8. Assay for phenotype or target gene expression 48 - 72 hours after transfection</li> </ol>	<ol style="list-style-type: none"> <li>1. Prepare bEnd-3 cell suspension: <ol style="list-style-type: none"> <li>a. Trypsinize cells (0.05% Trypsin) for 3-5 minutes at 37°C</li> <li>b. Dilute in complete growth medium to 5 x 10<sup>4</sup> cells/ml</li> </ol> </li> <li>2. Prepare transfection complexes by mixing 40 µl of serum-free medium, 5.5 µl of transfection reagent, and <ul style="list-style-type: none"> <li>• 750 ng DNA (or mRNA), or</li> <li>• 30 nM - 50 nM of siRNA (or microRNA)</li> </ul> <i>*Referred to a final volume including growth medium</i> </li> <li>3. Incubate transfection complexes at RT for 15 - 30 minutes</li> <li>4. <u>Optional:</u> Add 2 µl of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity</li> <li>5. Plate 25,000 - 35,000 cells per well in 0.5 ml of complete growth medium (from step #1) into culture plate</li> <li>6. Add prepared transfection complexes (from step 3 or 4)</li> <li>7. Incubate cells at 37°C in a humidified CO<sub>2</sub> incubator</li> <li>8. Assay for phenotype or target gene expression 48 - 72 hours after transfection</li> </ol>
<u>Optional:</u> Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 µl of Transfection Enhancer reagent 12-24 hours after transfection	<u>Optional:</u> Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 µl of Transfection Enhancer reagent 12-24 hours after transfection
If the viability of bEnd-3 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant	If the viability of bEnd-3 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant

## Scaling Up or Down Transfections:

Culture Vessel Surface Area (cm <sup>2</sup> )	Volume of Growth Medium (ml)	Transfection Reagent (µl)	Complex Condenser (µl)	Transfection Enhancer (µl)
96-well, 0.3 cm x cm	0.12	1.5	0.3	0.3
24-well, 2 cm x cm	0.5	5.5	2	2
12-well, 4 cm x cm	1	12	4	4
6-well, 10 cm x cm	3	35	12	12
60-mm, 20 cm x cm	5	60	20	20
10-cm, 60 cm x cm	15	180	60	60

## Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying bEnd-3 cell density and amount of transfection reagent. High passage of bEnd-3 cells and use of antibiotics (or growth factors) may require using larger volumes of bEnd-3 transfection reagent per reaction.

### Limited Use Label License:

The purchase of this product conveys to the purchaser the limited right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. This product is for research purposes only and is not for use in commercial applications of any kind. For information on obtaining additional rights, please contact Altogen Biosystems at [orders@altogen.com](mailto:orders@altogen.com).

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