TransfeX™ Transfection of Plasmid DNA into BT142 mut/- Cells

BT142 mut/- (ATCC® ACS-1018™) is a human neural suspension cell. ATCC achieved transfection efficiencies of ~40**%,** using the protocol described below.

General Considerations for using the TransfeX transfection reagent:

- All steps should be performed in a biosafety cabinet using proper aseptic technique.
- **Cell conditions.** Cells should be passaged at least once after thaw and the use of low-passage cells is recommended. Passage the cells 18-24 hours before transfection to ensure the cells are actively dividing and that they will be at the appropriate cell density at the time of transfection. Make sure that the cells are healthy and are ≥ 90% viable, prior to transfection.
- **Seeding density.** Cell density should be 50-80% confluent on the day of transfection. See specified seeding density in the individual protocols and in Table 1. *Note: Determine the optimal cell density for each cell type in order to maximize transfection efficiency*.
- **DNA purity.** Use highly purified plasmid preps that are free from phenol or other contaminants. Plasmid DNA preps that are endotoxin-free are desirable.
- **Presence of antibiotics and other inhibitors.** Antibiotics will inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added to cells grown in complete culture medium containing serum and low levels of antibiotics if required.
- **Complex formation conditions.** Prepare TransfeX Reagent and DNA complexes in serum-free growth medium. ATCC recommends using Opti-MEM I Reduced-Serum Medium to dilute the DNA before complex formation.

Materials required:

Material Required	Catalog No.
BT142 mut/-	ATCC® ACS-1018™
Option 1: NeuroCult NS-A Proliferation kit	Stem Cell Technologies Catalog No. 5751
Option 2: DMEM/F12 (1:1) with an additional 0.9% glucose	ATCC® 30-2006
4 mM L-glutamine	ATCC® 30-2214
25 µg/mL insulin	
100 µg/mL transferrin	
20 nM progesterone	
15 µM putrescine	
30 nM selenite	
TransfeX™	ATCC® ACS-4005

American Type Culture Collection
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Or contact your local distributor.

Opti-MEM® I Reduced-Serum Media	Life Techologies™ 31985-062
Plasmid DNA of interest (0.5µg/µL)	
Tissue culture plates and supplies	

Protocol:

The following protocol describes how to transfect plasmid DNA into BT142 mut/- cells using the TransfeX Reagent in a **single well of a 24-well plate.** The reaction may be scaled up as needed. Please refer to Table 1 for recommended reaction conditions for other dish or plate sizes.

A. Preparation of the cells for transfection

The day before transfection:

1. Count and measure cells for density and viability.
2. Plate **$1.0 \times 10^5 - 1.5 \times 10^5$** cells per well in 0.5 mL of complete growth medium (**DMEM + 10% FBS**). Cell density should be **50 - 80%** confluent on the day of transfection. **NOTE:** At this stage, neurospheres are dispersed into single cells (few neurospheres will remain but is not a concern).
3. Incubate cells overnight at **37°C** with **5% CO_2**.

B. Preparation of the DNA:TransfeX transfection complexes

1. Warm TransfeX, plasmid DNA, and Opti-MEM I Reduced-Serum Medium to room temperature and vortex gently to mix.
2. Pipette 100 µL Opti-MEM I Reduced-Serum Medium into a sterile microcentrifuge tube.
3. Add 4.0 µL (0.5 µg/µL) plasmid DNA.
4. Mix thoroughly with gently pipetting.
5. Add 6.0 µL TransfeX Reagent to the diluted DNA mixture. *Note: Do not let the pipette tip or the reagent come into contact with the sides of the plastic tube.*
6. Mix TransfeX:DNA complexes thoroughly using either a vortex or by pipetting briefly.
7. Collect contents at bottom of the tube using a mini-centrifuge.
8. Incubate TransfeX:DNA complexes at room temperature for 15 minutes.

C. Addition of DNA:TransfeX transfection complexes to the cells

1. Distribute the complexes to the cells by adding the complexes drop-wise to different areas of the well.
2. Gently rock the culture vessel back and forth and from side to side to evenly distribute the TransfeX:DNA complexes.

D. Post-Transfection Handling

1. Incubate for **approximately 3** hours.
2. 3.0hrs post transfection, transfer the transfected cells from the 24-well plate into a six-well plate.

3. Into the 24-well plate, add 500.0µL OPTI-MEM I/well to wash off any remaining cells. Transfer remaining suspension cells into the 6-well plate and add 1.0mL of the OPTI-MEM I so that the final volume is equivalent to 2.0mL/well.

4. Incubate in 37°C with 5% CO_2

E. Collection of data (GFP Expression)

1. At 24.0hrs post transfection, determine GFP expression by fluorescence microscope and flow cytometry.
2. Add 1.0mL fresh complete growth media/well.
3. Continue incubation to collect day 2-7 data.

NOTE: When the neurospheres begin to form large spheres (larger than 200µm), break spheres by pipetting repeatedly with a P1000 micropipette.

Table 1: Recommended Reaction Conditions for different size culture vessels.

Culture Vessel	24 well plate	12 well plate	6 well plate	10 cm dish
Surface area	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2
Complete Growth Medium	0.5 mL	1.0 mL	2.0 mL	4.0 mL
Opti-MEM I Reduced Serum Medium	100 µL	200 µL	400 µL	0.8 mL
DNA (0.5 µg/µL stock)	0.5 µg	1.0 µg	2.0 µg	4.0 µg
TransfeX Reagent	6.0 µL	12.0 µL	24.0 µL	48 µL

Note: Always include a control condition consisting of an empty vector plasmid or a plasmid expressing GFP.

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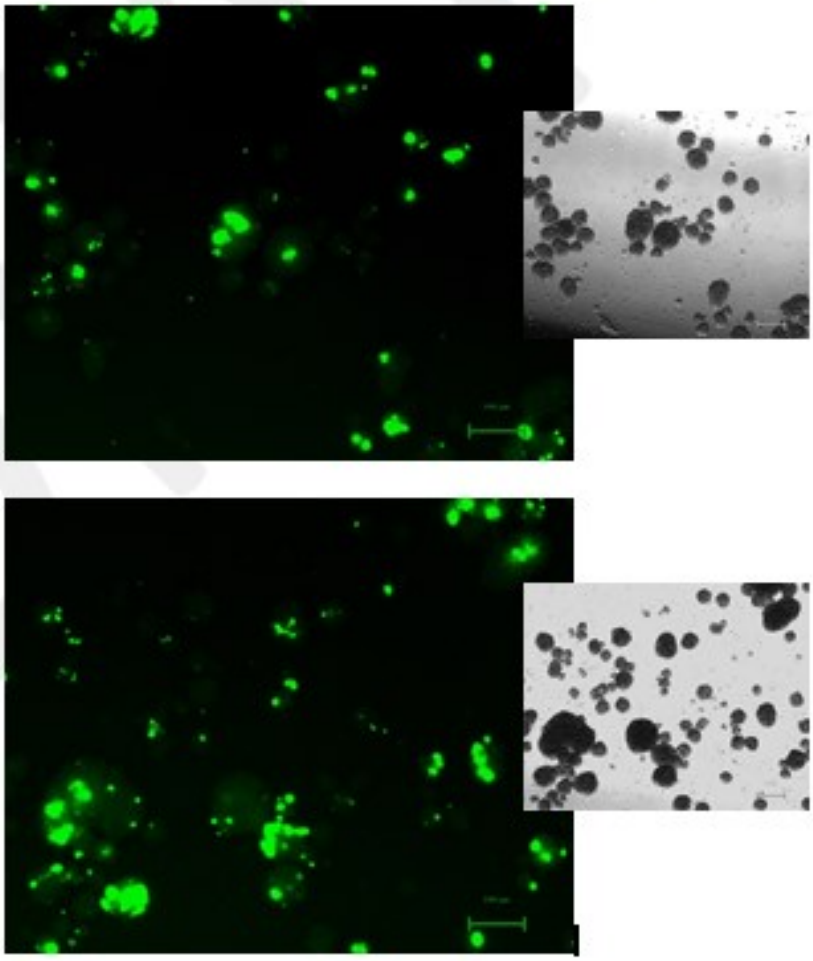

Transfection efficiency of TransfeX reagent on BT142 mut/-. Cells were transfected with EF1α-eGFP empty vector at 0.5 µg DNA with 6.0 µL of reagent in Opti-MEM I Reduced Serum Media. No significant signs of cellular toxicity were observed.

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