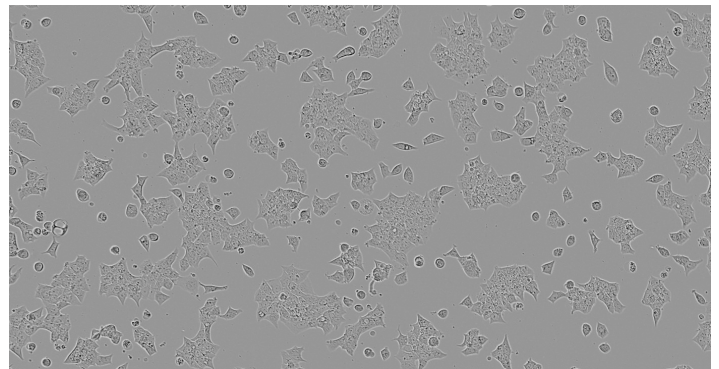


BT-474

Breast cancer cells

Complete growth medium

| Component | Cat. No. |
|---|----------|
| Gibco™ DMEM with GlutaMAX™ Supplement | 10566016 |
| 10% Gibco™ FBS | A3160401 |
| 25 mM Gibco™ HEPES (1 M) | 15630080 |
| 1.0 mM Gibco™ Sodium Pyruvate (100 mM) | 11360070 |
| 0.1 mM Gibco™ MEM Non-Essential Amico Acids Solution (100X) | 11140050 |



Proper culture techniques and procedures are an essential part of ensuring successful transfection. Subculturing, also referred to as passaging, is the removal of medium and transfer of cells from a culture into fresh growth medium, in order to propagate the cells.

Passaging

- Maintain cells in T-75 flasks.
- Use Gibco™ TrypLE™ dissociation reagent.
- Passage cells every 3–4 days to ensure that they do not enter senescence.
- Transfection of cells should be performed only between passages 5 and 25 post-thaw.
- If designing an experiment that involves transfection, ensure that setup coincides with a cell passage.
- Plate cells for transfection only 1 day before the experiment.

Seeding cells for transfection

- The day before transfection, dissociate cells that are 80–90% confluent in a T-75 flask.
- Count the cells using standard trypan blue exclusion.
 - **Important:** The cell number and concentration determined can vary significantly depending on what method is used for counting; it is important to be consistent and use a single method throughout an experiment.
- The cell culture must have >90% viability and be 60–70% confluent on the day of transfection.
 - **Important:** If cells are not at the right confluence, do not wait until the next day to perform transfection, as this can significantly affect transfection efficiency.
- Seed 13.5×10^4 cells in 500 μ L growth medium for a single well of a 24-well plate.

Transfection protocol

Transfection components

| Component | Cat. No. |
|---|----------|
| Thermo Scientific™ Nunc™ 24-Well Cell-Culture Treated Multidishes | 142475 |
| Invitrogen™ Lipofectamine™ 3000 Transfection Reagent | L3000008 |
| Gibco™ Opti-MEM™ I Reduced Serum Medium | 31985062 |

On the day of transfection, which should be 1 day following cell plating, perform the following steps, which have been optimized for a single well of a 24-well plate using Invitrogen™ Lipofectamine™ 3000 Transfection Reagent:

| Step | Tube | Complexation components | Amount per well (24-well) |
|------|--|---|---------------------------|
| 1 | Tube 1 | Opti-MEM™ I medium | 25 μ L |
| | | Lipofectamine 3000 reagent | 1 μ L |
| 2 | Tube 2 | Opti-MEM I medium | 25 μ L |
| | | DNA amount (DNA concentration should be 0.5–5 μ g/ μ L) | 250 ng |
| | | P3000™ reagent | 0.5 μ L |
| 3 | Add tube 2 solution to tube 1 and mix well | | |
| 4 | Incubate mixture from step 3 at room temperature for 10–15 min | | |
| 5 | Add 50 μ L of complex from step 4 to cells; gently swirl plate to ensure homogeneous distribution of complex to the entire well | | |

Transfection efficiency analysis

At 48 hr following transfection of a GFP reporter construct, cells were evaluated via microscopy and flow cytometry. To assess transfection efficiency, cells were first visualized via fluorescence microscopy for qualitative assessment of protein expression, morphology, and viability (Figure 1). Cells were then prepared for flow cytometry by aspirating the medium and replacing it with 250 μ L of a 7:3 mixture of TrypLE reagent: 1X DPBS. Cells were incubated at 37°C for 15 min and then pipetted up and down to ensure single cells for flow cytometry analysis.

Tips and tricks

- Decreasing the serum content of the culture medium (to <10%) at the time of transfection is acceptable, but replace with complete growth medium within 4–24 hr posttransfection.
- Antibiotics can be used during transfection.
- Prior to flow cytometry, visualize cells under a brightfield microscope to verify dissociation following incubation with TrypLE reagent.
- If cells have not fully detached after 15 min, place them back in the incubator for another 10 to 15 min.
- Gently tap on the side of the plate to aid in cell detachment when observing only partial attachment or detachment time is taking more than 30 min.

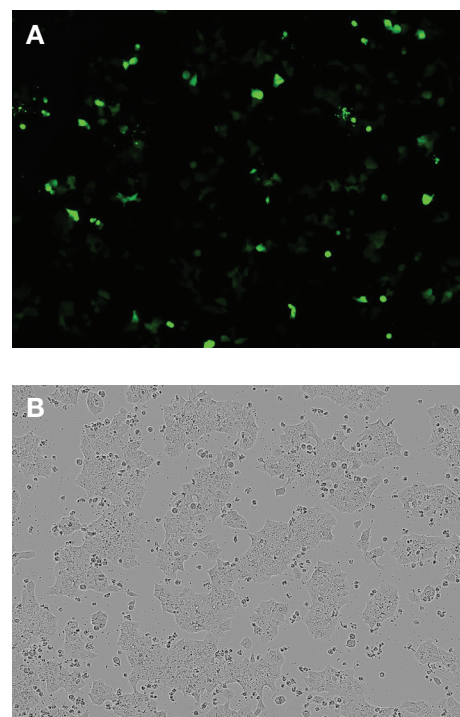


Figure 1. Posttransfection analysis of cells. (A) Fluorescence and (B) brightfield images demonstrating 52–55% transfection efficiency.

Scaling up or down Lipofectamine 3000 reagent transfections

Use the following table to scale the volumes for your transfection experiment. The most common sizes are listed below.

| Culture vessel | Multiplication factor* | Shared reagents | | DNA transfection | | | siRNA transfection | |
|----------------|------------------------|-----------------|--------------------------------|------------------|---------------|------------------------------|--------------------|------------------------------|
| | | Growth medium | Opti-MEM medium for complexing | DNA | P3000 reagent | Lipofectamine 3000 reagent** | siRNA | Lipofectamine 3000 reagent** |
| 96-well | 0.2 | 100 µL | 2 x 5 µL | 50 ng | 0.1 µL | 0.2 µL | 3 pmol | 0.3 µL |
| 48-well | 0.5 | 250 µL | 2 x 12.5 µL | 0.125 µg | 0.25 µL | 0.5 µL | 7.5 pmol | 0.75 µL |
| 24-well | 1 | 500 µL | 2 x 25 µL | 0.25 µg | 0.5 µL | 1 µL | 15 pmol | 1.5 µL |
| 12-well | 2 | 1 mL | 2 x 50 µL | 0.5 µg | 1 µL | 2 µL | 30 pmol | 3 µL |
| 6-well | 5 | 2 mL | 2 x 125 µL | 1.25 µg | 2.5 µL | 5 µL | 75 pmol | 7.5 µL |
| 60 mm | 11.05 | 5 mL | 2 x 250 µL | 2.75–5.5 µg | 5.5–11 µL | 11 µL | 166 pmol | 17 µL |
| 10 cm | 28.95 | 10 mL | 2 x 500 µL | 7–14 µg | 14–28 µL | 28.95 µL | 434 pmol | 43 µL |
| T-75 | 39.47 | 15 mL | 2 x 750 µL | 10–20 µg | 20–40 µL | 39.5 µL | 592 pmol | 59 µL |
| T-175 | 92.11 | 35 mL | 2 x 1.75 mL | 23–48 µg | 46–90 µL | 92.1 µL | 1,382 pmol | 138 µL |

* After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.

** Optimum amount needed is determined from the protocol for Lipofectamine 3000 Transfection Reagent.

For your convenience, the essential components of this protocol are now available in the Gibco™ Breast Cancer Starter Kit. The kit includes: basal medium, FBS, Lipofectamine 3000 reagent, Opti-MEM medium, and TrypLE reagent. The kit is available at thermofisher.com/cancercellculture

Find out more at thermofisher.com/3000