PHOREUS® BIOTECH

Transfecting Nucleic Acid into Eukaryotic Cells Using BAPtofect™-25 Reagent

Introduction

BAPtofect™-25 Reagent is a proprietary animal-free formulation for the transfection of Nucleic Acid into eukaryotic cells with minimal toxicity. This reference provides a recommended procedure to transfect DNA or RNA into eukaryotic cells using the BAPtofect™-25 reagent.

Guidelines for Transfection

Follow these important guidelines when transfecting DNA or RNA into eukaryotic cells when using the BAPtofect™-25 Reagent.

- The addition of antibiotics to cell culture medium during transfection may result in cell death. If you wish to use antibiotics during transfection, test the conditions before conducting transfection experiments.
- Maintain the same seeding density conditions between experiments. Use low-passage cells and make sure that the cells are healthy and are greater than 90% viable before transfection.
- Transfection can be performed both in the presence or absence of serum. Test serum-free medium for compatibility with BAPtofect™-25 Reagent
- We recommend Opti-MEM™ I Reduced Serum Medium (Gibco) to dilute the RNA or DNA and BAPtofect™-25 reagent.
- Visit or call Phoreus Biotech Technical Service for specialized transfection protocols.

Material Needed

Have the following reagents on hand before beginning:

- Cells to be transfected using standard cell growth propagation methods.
- Nucleic Acid of interest at 20-100ng/µl or higher concentration
- BAPtofect™-25 kit reagents – (store at 4°C or keep on ice until use)
- Opti-MEM Reduced Serum Medium
- Complete cell culture medium, (needed for the day after transfection).
- Appropriate tissue culture vessels and supplies

Use this procedure to transfect Nucleic Acid (NA) into Eukaryotic cells cultured in a well of an 8 well chamber slide. (Suggestions for other growth vessels are included below).

1. The day before transfection, collect cells by trypsinization or other method and count the cells.
2. Seed cells so that they reach 50 to 80% confluency by the time of transfection. Generally, dilute cells to 0.5 - 2 x 10^5 cells/ml in complete growth medium specific for the cell type and add an appropriate volume (150 – 200ul) to each well of the chamber slide (or other vessel).
3. Incubate the cells overnight in a 37°C, humidified incubator with 5% CO_2.
4. Prepare BAPtofect™-25 reagent transfection mixture immediately before transfection.
   - Bring the BAPtofect™-25 reagent and the OLL10 reagent to Room temperature

- Dilute target NA to have a concentration of between 20 - 100 ng/μl in Molecular Biology Grade Water.
- Aliquot 194μl of Opti-MEM I reduced-serum medium (FBS can be included at 1-2% but is not necessary) to a nuclease-free tube.
- Add 2 μl of BAPtofect™-25 reagent to the OptMEM I medium in the tube. Gently pipet up and down to completely mix.
- Add 2 μl of diluted NA (20-100 ng/μl) to the BAPtofect™-25/ OptiMEM mixture. Gently pipet up and down and incubate for 15 minutes at room temperature.
- After 15 minutes of incubation, add 2 μl of the OLL10 reagent to the mixture and incubate another 15 minutes at room temperature.
- This is the prepared Transfection Solution for 1 well of an 8 well chamber slide.

5. Prepare cells for transfection.
   A. At the time/day of transfection remove the growth medium from the cells.
   B. Gently wash the cells once with Opti-MEM or DPBS and discard.
   C. Gently add 150μl of the prepared BAPtofect™-25 transfection solution to the well with cells to be transfected.
   D. Incubate at 37°C in a humidified, 5% CO_2 incubator for 2-4 hours. Incubation time may vary depending on the purpose of experiments.
   E. After 2-4 hours of incubation, remove the transfection solution.
   F. Add 200μl of complete growth medium to each well that is being transfected. Place cells back into the incubator.
   G. After 24-72 hours of incubation conduct the detection assay for the experiment.

Table 1. Recommended starting points

Culture Vessel	Surface Area cm^2	Seeding Density cells/well	Vol of TM/well
8 well CS		5×10^4	200μl
4 well CS		1×10^5	300μl
2 well CS		2×10^5	400μl
96 well plate	0.3	2×10^4	100μl
48 well plate	1	1×10^5	300μl
24 well plate	2	2×10^5	400μl
12 well plate	4	4×10^5	500μl
6 well plate	10	5×10^5	600μl

CS = Chamber Slide TM = transfection mixture

Table 2. Recommended starting volumes

Component	8 well CS/well	4 well CS/well	2 well CS/well	96 well plate/well	48 well plate/well	24 well plate/well	12 well plate/well	6 well plate/well
Nucleic Acid	2µl	3µl	4µl	1µl	3µl	4µl	5µl	6µl
BAPtofect^{TM}-25	2µl	3µl	4µl	1µl	3µl	4µl	5µl	6µl
OLL 10	2µl	3µl	4µl	1µl	3µl	4µl	5µl	6µl
SF Medium	194µl	291µl	388µl	97µl	291µl	388µl	485µl	582µl
Total Vol	200µl	300µl	400µl	100µl	300µl	400µl	500µl	600µl

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