



Biosystems™

*The Transfection &
Gene Expression Experts*

Cementoblast Avalanche® Transfection Reagent

Cat. No. EZT-OCCM-1

Size: 0.5 ml
1.5 ml

Store at 4°C

Cell Line Information:

Cementum, a mineralized tissue lining the surface of the tooth root, is required for formation of a functional periodontal ligament attachment during development. Additionally, during regeneration of tissues after disease, cementum is thought to play a critical role in the reparative process.

Cementoblasts play a central role in periodontal development and regeneration, and in particular the formation of root cementum. Cementoblasts were first isolated from OC-TAG transgenic mice in Martha Somerman lab in University of Michigan, Ann Arbor. These mice contain a construct that employed the SV-40 T antigen under the direction of the osteocalcin (OCN) promoter. In this situation, when cells are isolated from the tooth root surface of OC-TAG mice, only those cells that express osteocalcin (i.e., cementoblasts) are capable of surviving serial passages in vitro. The immortalized cementoblasts obtained in this manner were subcloned, and one clone was designated as OCCM-30. These cells were shown to exhibit the same properties as comparable cells found in vivo, i.e., they expressed high levels of cementum-associated markers, such as BSP and OCN, and promoted biomineralization in vitro and in vivo.

The Transfection Reagent:

Cementoblast (OCCM-30 cell) Avalanche® Transfection Reagent is a new, proprietary solution specifically designed for transfection on cementoblasts (OCCM-30 cells). The proprietary formulation of lipids and polymers ensures the highest possible transfection efficiencies and viabilities for cementoblasts (OCCM-30 cells).

Features:

- Unique formulation-maximize transfection performance in cementoblasts (OCCM-30 cells). More than 90% transfection efficiency as indicated by GFP positive cells in flowcytometry analysis.
- Extremely gentle to cells
- Much less reagent needed for each transfection: 0.5 ml is able to transfect about 1000 wells of 24-well plate
- Deliver single or multiple plasmids
- Compatible with serum
- Compatible with transfection in any plate formats

BEFORE YOU START:

Important Tips for Optimal Transfection

1. Prepare high-quality plasmid DNA at 0.5–5 µg/µl in deionized water or TE buffer. A GFP (green fluorescent protein) plasmid can be used to determine transfection efficiency.
2. Use Opti-MEM[®] I Reduced Serum Medium (Life Technologies) or regular DMEM without serum to make The Reagent and nucleic acid mix (Only Opti-MEM[®] I will be mentioned in the remaining parts of the protocol for simplification purpose). Do not use NaCl₂ solution or PBS.
3. Maintain the same seeding conditions between experiments. Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
4. The Reagent is extremely gentle to cells. However, transfection process will impose stress on cells, no matter what type of transfection methods you use. The trick is to get the balance between transfection efficiency and cell viability. Increasing the number of cells plated per well or decreasing DNA/The Reagent amount will minimize the effect of transfection on cell growth and viability. With careful adjustment, as described in page 3 and 4, this can be achieved while keeping the highest transfection efficiency.
5. Don't use antibiotics in the culture medium during the first 24 hours of transfection.

Protocols

1 DNA Transfection

1.1 Cell Seeding

For optimal DNA transfection conditions, we recommend using cells which are 70% to 90% confluent at the time of transfection. Typically, for experiments in 24-well plates, 50,000-80,000 cells are seeded per well in 0.5 ml of cell growth medium **without antibiotics** 24 h prior to transfection. For other culture formats, refer to Table 1.

Table 1. Recommended number of cells to seed the day before transfection

| Culture vessel | Number of cells to seed | Surface area per well (cm ²) | Volume of medium per well to seed the cells (ml) |
|----------------------------------|--------------------------------------|--|--|
| 96-well | 7,500-10,000 | 0.3 | 0.1 |
| 24-well | 50,000-80,000 | 1.9 | 0.5 |
| 12-well | 80,000-150,000 | 3.8 | 1 |
| 6-well/35 mm | 150,000-250,000 | 9.4 | 2 |
| 60 mm/flask 25 cm ² | 250,000-800,000 | 25-28 | 5 |
| 100 mm/flask 75 cm ² | 1x10 ⁶ -2x10 ⁶ | 75-78.5 | 10 |
| 150 mm/flask 175 cm ² | 2x10 ⁶ -5x10 ⁶ | 153-175 | 25 |

1.2 DNA Transfection on cementoblasts

Due to cell culture variations and passage number differences, cementoblasts from different sources may have different sensitivity to The Reagent. If this is the first time that you are using The Reagent on your cementoblasts, follow the following procedures and Figure 1 for fine-tuning optimization (**The fine-tuning optimization procedures are extremely important for successful transfection. The amount of The Reagent needed for maximum transfection on cementoblasts from different sources may differ dramatically**).

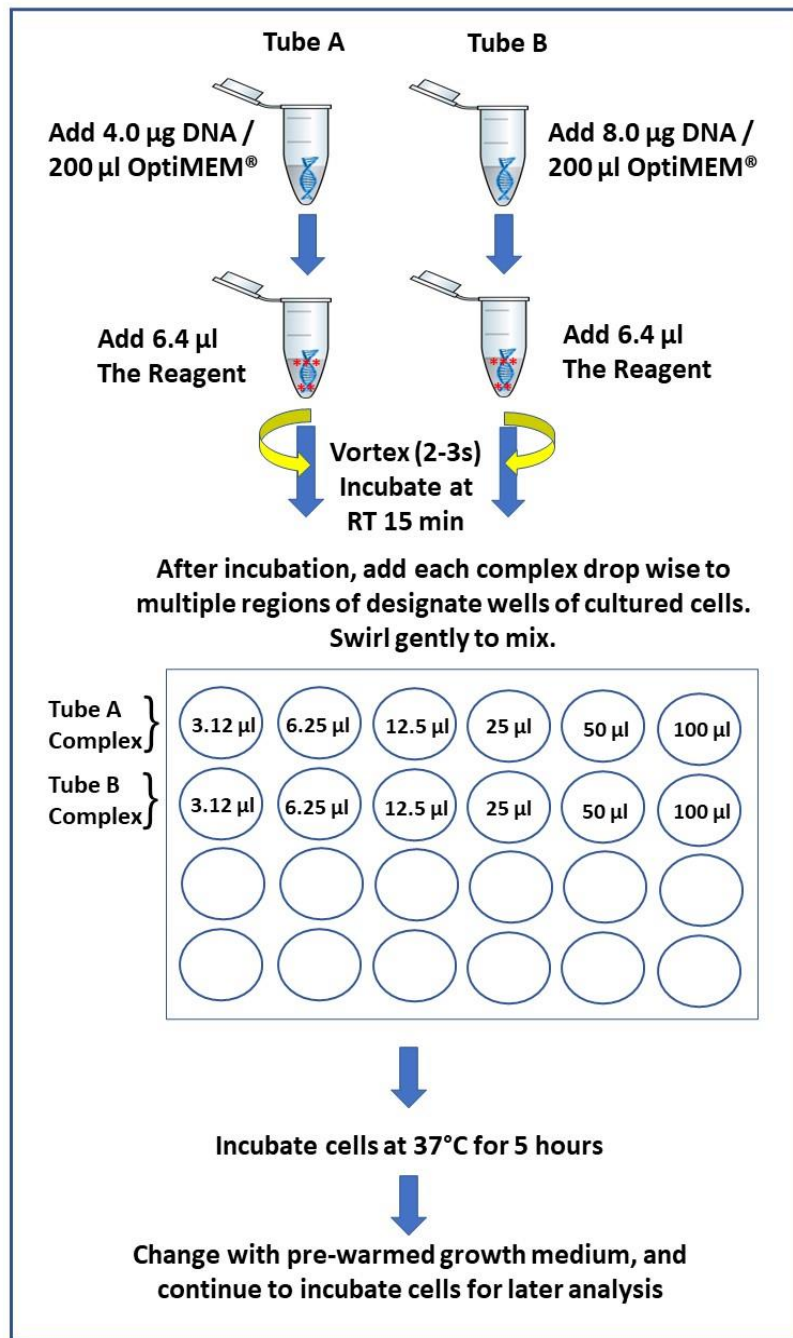
As an example, the following procedures and Figure 1 are for fine-tuning optimization on 24-well plate:

Figure 1.

1. Bring The Reagent and serum-free medium (OptiMEM®I) to room temperature before starting.
2. Add 200µl of OptiMEM®I into two 1.5ml tubes (Tube A and B).
3. Add 4.0 µg of DNA to Tube A and 8.0 µg of DNA to Tube B.
4. Mix The Reagent prior to preparing complexes. Add 6.4 µl of The Reagent to both Tube A and Tube B containing 200µl of different concentration of DNA solution (20.0 µg/ml and 40.0 µg/ml respectively). Vortex for 2-3 seconds. Incubate each tube for 15 minutes at room temperature.
5. After incubation, add 3.12, 6.25, 12.5, 25, 50, and 100µl of DNA/The Reagent complexes dropwise directly to the corresponding wells of the 24-well cell culture plate (See Figure 1). Swirl plate gently.
6. Incubate the cells at 37°C in a CO2 incubator 5 hours.
7. Change growth medium, and continue to culture in the incubator.
8. Expression of reporter gene activity should generally be assessed at 20-48 hours post-transfection. GFP expression is maximal at 40-48 hours post-transfection.

After you have completed the fine-tuning optimization steps, choose the amount of DNA and The Reagent that gave you the optimal balance of potency & low cytotoxicity (which

usually is the lowest dose that gave you the same high transfection efficiency as other higher doses did) for all of your future experiments on this specific cell type.



1.3 Scale Up or Down Transfections

Use Table 2 to scale the amount of DNA/The Reagent for your transfection experiment.

Table 2. Scaling Up or Down Transfection Instruction

| Culture Vessel | Multiplication factor* |
|----------------|------------------------|
| 96-well | 0.17 |
| 48-well | 0.50 |
| 24-well | 1.00 |
| 12-well | 2.00 |
| 6-well | 5.00 |
| 60-mm | 11.05 |
| 10-cm | 28.95 |
| T75 | 39.47 |

**After determining the optimum amount of DNA/The Reagent from the fine-tuning optimization procedures on the above 24-well plate, use the multiplication factor to determine the DNA and The Reagent amount needed for your new plate format.*

1.4 DNA Transfection on cells other than cementoblasts

Cementoblast Avalanche® Transfection Reagent can also be used on the following cells with high transfection efficiencies.

Caki-1 Cell, MDCK Cell, Vero Cell, 293 Cell, 293T/17 Cell

The above protocol usually provides satisfactory transfection efficiency with invisible cytotoxicity on the above cells. However, additional optimization may be needed for certain type of cells. Optimizations may include: cell density; the amount of DNA and The Reagent; DNA/The Reagent ratio, or incubation time for the mixture of The Reagent/DNA etc.

If you want to achieve the best transfection result for a specific type of cells, we recommend using the respective cell type/cell line specific Avalanche® transfection reagents. Those reagents have been optimized on both recipes and protocols, and have been proved to have the best transfection results for the respective cell lines or primary cells. You can easily find the respective Avalanche® Transfection Reagents specific for your cells by using the filters of our product list in EZ Biosystems website:

www.ezbiosystems.com.

2 siRNA Transfection

Though Cementoblast Avalanche® Transfection Reagent formulation was optimized for DNA transfection, it can also be used for siRNA transfection. The following are recommended protocol. Protocol optimization may be needed.

2.1 Cell Seeding

For optimal siRNA transfection conditions, we recommend using cells which are 50% confluent at the time of transfection. Typically, for experiments in 6-well plates, 100 000 to 150 000 cells are seeded per well in 2 ml of growth medium without antibiotics 24 h prior to transfection. For other culture formats, refer to Table 3.

Table 3. Recommended number of cells to seed the day before transfection.

| Culture vessel | Number of cells to seed | Surface area per well (cm ²) | Medium per well to seed the cells (ml) |
|---------------------------------|--|--|--|
| 24-well | 25,000-40,000 | 1.9 | 0.5 |
| 12-well | 50,000-80,000 | 3.8 | 1 |
| 6-well/35 mm | 100,000-150,000 | 9.4 | 2 |
| 60 mm/flask 25 cm ² | 200,000-500,000 | 25-28 | 5 |
| 100 mm/flask 75 cm ² | 0.5x10 ⁶ -1x10 ⁶ | 75-78.5 | 10 |

2.2 siRNA Transfection

We recommend using 10 to 50 nM siRNA (final concentration). The following conditions are given per well of a 6-well plate. For other culture format, please refer to Table 4.

1. Dilute 22 to 110 pmoles siRNA (final concentration: 10 to 50 nM) into 200 µl of Opti-MEM® Reduced-Serum Medium or regular high glucose DMEM without serum. Mix by vortexing.
2. Briefly vortex The Reagent, and add 1.0-5.0 µl into the diluted siRNA. Immediately vortex for 10 s.
3. Incubate for 15 min at RT.
4. Add the transfection mixture drop-wise into each well.
5. Gently put in incubator, and incubate at 37 °C CO2 for 5 hours.
6. Remove the media containing the transfection mixture, and add pre-warmed normal serum-containing fresh media. Continue to culture for 24 hours or more for analysis.

Table 4. siRNA transfection guidelines according to the cell culture vessel per well

| Culture Vessel | siRNA (pmole) 10 nM | siRNA (pmole) 50 nM | The Reagent (μl) | Opti-MEM or DMEM (μl) | Growth medium (ml) | Final Volume in the well (ml) |
|-------------------------------------|------------------------|------------------------|------------------|-----------------------|--------------------|-------------------------------|
| 24-well | 5.5 | 27.5 | *0.2-1.0 | 50 | 0.5 | 0.55 |
| 12-well | 11 | 55 | *0.4-2.0 | 100 | 1 | 1.1 |
| 6-well/ 35 mm | 22 | 110 | 1.0-5.0 | 200 | 2 | 2.2 |
| 60 mm/ flask 25 cm ² | 44 | 220 | 2.3-11.5 | 400 | 4 | 4.4 |
| 100 mm/ flask 75 cm ² | 121 | 605 | 5.8-29 | 1100 | 11 | 12.1 |

* Dilute The Reagent 1:5 with H₂O prior application (4 μl reagent + 16 μl H₂O), and then use 5 times of volume for accurate pipetting.

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