

To find the ideal conditions, VeroFect must be tested at ratios **1  $\mu\text{L}/\mu\text{g}$** , **2  $\mu\text{L}/\mu\text{g}$**  and **3  $\mu\text{L}/\mu\text{g}$**  ( $\mu\text{L}$  of VeroFect /  $\mu\text{g}$  of DNA). For the DNA quantity, we suggest **0.25  $\mu\text{g}$**  per well in 96-well, **0.5  $\mu\text{g}$**  per well in 24-well and **2  $\mu\text{g}$**  per well in 6-well.

**Seed cells to be at 70% confluent the day of transfection\***

1



**Prepare 3 identical tubes of DNA**

2



**96 well plate**

**24 well plate**

**6 well plate**

0.25 $\mu\text{g}$  in 25 $\mu\text{L}$  of serum-free medium or buffer\* X 3

0.5 $\mu\text{g}$  in 50 $\mu\text{L}$  of serum-free medium or buffer\* x 3

2 $\mu\text{g}$  in 100 $\mu\text{L}$  of serum-free medium or buffer\* x 3

**Prepare 3 tubes of VeroFect (with 3 different amounts of reagent)**

3



**96 well plate**

**24 well plate**

**6 well plate**

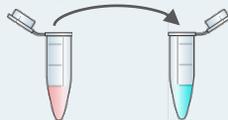
0.25 $\mu\text{L}$ /0.5 $\mu\text{L}$ /0.75 $\mu\text{L}$  in 25 $\mu\text{L}$  of serum-free medium or buffer\*

0.5 $\mu\text{L}$ /1 $\mu\text{L}$ /1.5 $\mu\text{L}$  in 50 $\mu\text{L}$  of serum-free medium or buffer\*

2 $\mu\text{L}$ /4 $\mu\text{L}$ /6 $\mu\text{L}$  in 100 $\mu\text{L}$  of serum-free medium or buffer\*

**Mix each tube of DNA (step 2) to each tube of VeroFect (step 3)**

4



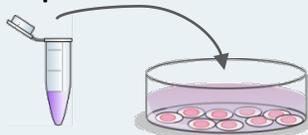
**Incubate 20 min at room temperature**

5



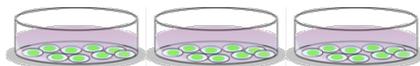
**Distribute each mix dropwise onto the cells to insure uniform distribution**

6



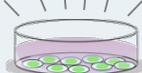
**Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression\***

7



**Choose the best ratio DNA:VeroFect**

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**\*NOTES:**

- (1) Of course the conditions provided above might required some further optimizations depending on your cells, DNA, RNA, etc...
- (2) For Vero cells, 24h before transfection seed the cells in a 96-well plate, 24-well plate or 6-well plate in respectively 150  $\mu$ L, 400  $\mu$ L and 2 mL of complete culture medium.
- (3) Allow reagents to reach RT and gently vortex them before forming complexes .
- (4) **Medium or buffer without serum & supplement** must be used for the DNA/VeroFect complexes preparation. Culture medium such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.
- (5) For doses of VeroFect less than 1 $\mu$ L, dilute the reagent with deionized water.
- (6) For some cells, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium.

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(781) 686-1631

[info@bocascientific.com](mailto:info@bocascientific.com)