ViaFect™ Transfection Reagent

INSTRUCTIONS FOR USE OF PRODUCTS E4981, E4982 AND E4983

Transfection Protocol for 96-Well Plate

Preparing the ViaFect™ Transfection Reagent

1. Before use, allow ViaFect™ Transfection Reagent to reach room temperature.
2. Mix by inverting.

General Transfection Protocol

1. To prepare transfection complexes: To a sterile tube or U- or V-bottom plate, add 90–99µl of serum-free medium prewarmed to room temperature such that the final volume after adding DNA is 100µl. Add 1µg of plasmid DNA, and mix. For a 3:1 ViaFect™ Transfection Reagent:DNA ratio, add 3µl of ViaFect™ Transfection Reagent, and mix. For other ratios, consult Table 1.

   Notes:

   - It is best to dispense the ViaFect™ Transfection Reagent directly into the media.
   - If cell toxicity is observed, using less DNA may reduce toxicity and yet maintain high transfection efficiency. This can be optimized for each cell line.

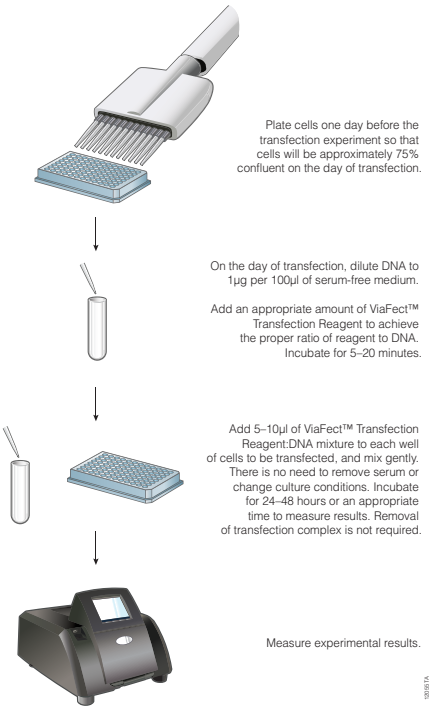

Table 1. Optimization of Transfection Conditions Using Varying Ratios of ViaFect™ Transfection Reagent:DNA.

	Ratio of ViaFect™ Transfection Reagent:DNA				
	1.5:1	**2:1**	**3:1**	**4:1**	**6:1**
Medium to a final volume of	100µl	100µl	100µl	100µl	100µl
DNA amount	1µg	1µg	1µg	1µg	1µg
Volume of ViaFect™ Transfection Reagent	1.5µl	2µl	3µl	4µl	6µl
These volumes are sufficient for 20 wells (5µl/well) or 10 wells (10µl/well) of a 96-well plate for each ratio.					

2. Incubate the transfection complexes for 5–20 minutes at room temperature.
3. Add 5–10µl of transfection complex per well to a 96-well plate containing 100µl of cells in growth medium. Mix gently. Return cells to the incubator.
4. Measure transfection efficiency using an assay appropriate for the reporter gene used. For transient transfection, cells are typically assayed 24–48 hours after transfection.

Additional protocol information in Technical Manual #TM409, available online at:
www.promega.com/protocols

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