

General Troubleshooting Tips

Symptoms	Possible Causes	Comments
No transfection or low transfection efficiency	Poor quality DNA	The DNA should be purified on CsCl gradients or equivalent methods. The $A_{260}:A_{280}$ ratio of the DNA should be 1.8 or greater.
Variable transfection in replicate cultures experiments	Cells are contaminated with <i>Mycoplasma</i>	Test cultures for <i>Mycoplasma</i> contamination. Destroy contaminated cultures and start a new culture from a fresh stock.
	Suboptimal growth of cells	Transfection efficiency may decrease if cells have been passaged for many generations. Start a fresh culture from cell stocks that were frozen at an early passage. Some cells, particularly lymphocytes, will exhibit variability in transfection efficiency if they are left in culture beyond 1-2 weeks.
	Variable cell density	Maintain a consistent cell density at the time of transfection for each experiment.

Cationic Lipid Reagent Troubleshooting

Symptoms	Possible Causes	Comments
No transfection or low transfection efficiency	Charge ratio of reagent to DNA is sub-optimal	Optimize the reagent to DNA charge ratio. Charge ratios of 1:1, 2:1, 3:1 and 4:1 work well for many cell lines, but ratios outside this range may be optimal for a particular cell type or application.
	Excessive cell death	Decrease the time of exposure of the cells to the reagent. Lower the amount of input DNA and cationic lipid reagent, while holding the charge ratio constant. Increase cell density for the transfection step. Remove cationic lipid reagent/DNA mixture from the cells after the transfection period and prior to adding complete medium.

Calcium Phosphate Transfection Troubleshooting

Symptoms	Possible Causes	Comments
No transfection or low transfection efficiency	Poor precipitate formation	<p>The CaCl₂/DNA and 2X HBS solutions should be at room temperature (22-25°C) when they are mixed. Higher or lower temperatures for precipitate formation can lead to decreased transfection efficiency.</p> <p>The addition of CaCl₂/DNA to the 2X HBS solution should be performed dropwise and with continuous mixing.</p> <p>The concentration of DNA can affect the size of the precipitate. Low amounts of DNA (less than 1µg) can be supplemented with sheared carrier DNA such as salmon or herring sperm DNA. However, there are conflicting reports in the literature as to the efficacy of adding carrier DNA (80,87).</p> <p>The precipitate should be added dropwise around the dish to the medium bathing the cells, and the medium should be mixed thoroughly at the end of the addition. This helps to evenly distribute the precipitate and avoid the localized acidification of cells.</p> <p>After the addition of the calcium phosphate precipitate to the cells, the pH of the medium should be between 7.2 and 7.4. The CO₂ concentration in the incubator should be maintained at an appropriate level (generally 5-10%, depending on the composition of the culture medium).</p>
	pH not optimal	<p>The pH of the HBS solution should be 7.1. A large volume of added DNA in Tris buffer could change the pH. The DNA should be resuspended in water, 1mM Tris or, if present in 10mM Tris, should be fairly concentrated so that a relatively small volume of the DNA solution is added to the HBS.</p> <p>pH of the 2X HBS may have changed on storage. Check the pH and adjust it to 7.1 if necessary.</p>

DEAE-Dextran Transfection Troubleshooting

Symptoms	Possible Causes	Comments
No transfection or low transfection efficiency	Excessive cell death	<p>Decrease the concentration of DEAE-Dextran or shorten the time during which the cells are exposed to DEAE-Dextran.</p> <p>Decrease the time of exposure to chloroquine.</p> <p>Certain types of cells that are very sensitive to DEAE-Dextran toxicity, such as primary cell cultures, may require a higher cell concentration at the time of transfection.</p> <p>For some cell lines, lower concentrations of DNA can be used for standard DEAE-Dextran transfections compared to calcium phosphate transfection. Establish a dose-response curve to determine the optimal DNA concentration to use.</p>

