BioResearch

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Essentials for Preparing a Transfection Experiment – Plasmid DNA

Technical Reference Guide

Preparation and Quality

The quality of DNA used for transfection plays a central role for the success of the experiment.
We strongly recommend the use of high quality products for plasmid purification, e.g., Qiagen® EndoFree® Plasmid Kits. The purified DNA should be resuspended in sterile deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) before use. It has been demonstrated that DNA which was not purified via an endotoxin-free method can result in poor cell viability for some cell types. The same effect can be observed when endotoxin is added to clean DNA preparations. We do not recommend using phenol:chloroform or other organics in the preparation of the DNA as these are toxic to living cells and very difficult to remove completely. For cells which are sensitive to activation by lipopolysaccharides, such as monocytes, macrophages and dendritic cells, an additional purification step via PEG precipitation is helpful. DNA should be dissolved in water. To 100 µl DNA in water, add 750 µl 5.0 M NaCl and 750 µl 40 % w/v PEG 8000. Mix the contents of the tube by inverting several times and incubate on ice for 1 hour. Spin at top speed in a microcentrifuge for 15 minutes at 4° C. Remove supernatant, dissolve pellet in 100 µl water and repeat PEG precipitation. Carefully remove supernatant. Rinse the pellet with 500 µl ice cold 70 % ethanol. Spin 3 minutes. Remove supernatant. Air dry the pellet and resuspend in 20 µl sterile water or TE (adapted from Molecular Cloning: A Laboratory Manual (Third Edition) by Joseph Sambrook, Peter MacCallum Cancer Institute, Melbourne, Australia; David Russell, University of Texas Southwestern Medical Center, Dallas).

Measuring Quality and Concentration of DNA

DNA purity should be measured by the ratio of absorbance (A) at 260 to 280 nm. The A260:A280 ratio should be at or above 1.6 for transfection use. Additionally, the intactness of the plasmid should be verified on an agarose gel. At least 90 % of the DNA should be in the supercoiled conformation and no degradation products should be visible. To determine concentration, measure the absorbance at a wavelength of 260 then calculate as follows: A260 x 50 µg/ml x dilution factor = DNA concentration. Be sure that the dilution used is in the linear range of the spectrophotometer, usually an OD of 0.1–1.0. If using a microcuvette with a path length of less than 1 cm, it will be necessary to multiply by the factor to convert to the OD of a 1 cm path. For example, the path length of the 5 µl cuvette is only 0.5 mm or 1 / 20 cm, so would require multiplying the above formula by 20 to get the concentration.

How Much DNA?

Nucleofection™ : Gene transfer efficiency can also be affected by the amounts of DNA. For Nucleofection™ of most cell types, we start with 1–2 µg DNA per 100 µl reaction with our pmaxGFP™ Vector which is ~4 kb in size. For larger constructs, it may be necessary to add higher amounts of DNA, so we recommend titrating the DNA to see if increasing amounts are helpful. The plasmid amounts can be increased up to 10 µg per sample or more in some cases. However, certain cells are sensitive to DNA and, in those cases, more DNA will result in increased mortality of the cells. If the Optimized Protocol for a cell type recommends using less than 2 µg of pmaxGFP™ Vector, those cells are likely DNA sensitive.

Note
The DNA concentration should be such that no more than 10 µl of substrate per 100 µl reaction is added in order to not dilute the Nucleofector™ Solution too far or exceed the tolerance of the cuvette, which could result in an error on the device.

What If My DNA Is Too Diluted?

In order to keep the total DNA volume to add to a Nucleofection™ Reaction in the appropriate range, it may be necessary to ethanol precipitate your DNA if it is too diluted. An ammonium acetate-based ethanol precipitation followed by two 70 % ethanol washes should ensure that there is minimal salt carryover. The procedure is to add 0.5 volumes of 7.5 M ammonium acetate and 2 volumes ethanol to the DNA in solution and mix well. Spin at full speed in a microcentrifuge for 15 minutes. Carefully remove supernatant. Rinse the pellet with a volume equal to the precipitation of ice cold 70 % ethanol. Spin for 5 minutes. Remove supernatant. Repeat. Airdry the pellet and resuspend in sterile water or TE. Generally an assumption of about 70 % recovery is good for determining the volume to resuspend. Then read A260 to confirm.

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