FAQ of GenJet™ In Vitro DNA Transfection Reagent

Question: How long time is required for complex formation after GenJet™ Transfection Reagent is added to the DNA-saline mix**:**

Answer: Our R&D scientists have determined that allowing the GenJet-DNA mix to sit around 10 min at room temperature is optimal for transfection.

Question: How many reactions do you get from one mL of GenJet™ Transfection Reagent?

Answer: The number of reactions depends on the type of culture vessel you are using. For 6-well plates, you get 60-120 transfections. For 24-well plates, you get 300-600 transfections.

Question: The protocol included with GenJet™ Transfection Reagent gives the procedure in 6-well plates, but I'm using 24-well plates. How do I scale this for 24-well plates?

Answer: The components of the reaction are scaled down to these amounts: sterile diluent: 50 µL, plasmid DNA: 0.6 µg, GenJet™: 2.4 µL. If you would like a detailed protocol for transfections in 24-well plates, please contact us at tech@signagenlabs.com

Question: What is the optimal GenJet™:DNA ratio?

Answer: Optimal results are obtained with a GenJet™:DNA ratio (µl of GenJet™ : µg of DNA) of 3:1. This means you use 3 µl GenJet™ for every 1 µg of plasmid DNA. To obtain this ratio, it's critical that the DNA concentration is accurately known. The DNA concentration should be calculated from the absorbance of a dilution in a spectrophotometer. One µg DNA in 100 µl has an absorbance of 0.2 at 260 nM.

Question: Is GenJet™ Transfection Reagent an effective reagent for the transfection of siRNA molecules?

Answer: Yes, we have observed successful transfection of siRNA molecules.

Question: Can I use antibiotics in the media when using GenJet™ Transfection Reagent?

Answer: Yes. Antibiotics do not interfere with GenJet™-mediated transfections.

Question: The recommended storage temperature for GenJet™ Transfection Reagent is room temperature. Can I store it at 4 °C instead?

Answer: GenJet™ is not temperature sensitive. It can be stored at room temperature, 4° C or -20° C, whichever is most convenient. Simply vortex/mix briefly before use, to insure that everything is in solution.

Question: What percentage serum is optimal for transfection?

Answer: We recommend starting with 2% serum in the media. Maximal transfection efficiencies are observed in the range of 0-3% serum. We have observed a moderate inhibitory effect on transfection with 5 % serum in the media.

Question: Is it necessary to perform a media exchange?

Answer: We suggest that a media exchange is performed 1-2 hours prior to transfection. It is not required to exchange the media after transfection with GenJet™. Toxicity is not generally an issue with GenJet™. However, some customers prefer to perform a media exchange because their cells are particularily sensitive to transfection-related toxicity. In those cases, the complete media with 10 % serum can be exchanged 4~5 hours post-transfection.

Question: Your literature states that you've used GenJet™ with COS cells, and it works very well. What is the transfection efficiency in COS?

Answer: We achieve above 80% efficiency in COS cells.

Question: What amounts should I use for transfection in T25 flasks?

Answer: For transfection in T25 flasks, use: sterile diluent (serum-free DMEM medium) 0.5 ml, DNA 6-12 µg and GenJet™ 24-48 µL. The culture volume should be 5 mL.

Question: Which variables are most important to evaluate, in an effort to optimize transfection efficiency in a given cell line?

Answer: The most critical parameters to evaluate are:

1. Plasmid DNA Quality: The DNA should have an A_{260}:A_{280} ratio of $\geq$ 1.8. The integrity of the DNA should also be assessed via diagnostic digests, using at least two different restriction enzymes. In addition, agarose gel electrophoresis of uncut plasmid should show that the plasmid prep being used for transfection is $\geq$ 90% supercoiled DNA.

2. GenJet™:DNA ratio: We recommend using an initial GenJet™:DNA ratio of 3:1. In determining the optimal ratio for your cell line of interest, try using ratios of 2:1, 3:1, 5:1, and 8:1.

3. Total DNA: Once an optimal GenJet™:DNA ratio has been established, keep this ratio constant and the overall transfection mix (GenJet™ + DNA + diluent) volume constant, while titrating the total amount of DNA and GenJet™ present in the transfection.

4. Cell Density: The cell density at the time of transfection is typically more critical in suspension cell culture transfections than adherent cells. It is recommended that an initial cell density of 10^6 cells/ml is used. To identify the optimal cell density at the time of transfection, it is recommended that an actively growing culture be used that shows $\geq$ 90% viability, and that densities of 5 x 10^5, 10^6, and 1.5 x 10^6 cells/ml are evaluated. When using cell lines and/or culture conditions that promote high density growth of your cells, transfecting at 2 x 10^6, 2.5 x 10^6, and 3 x 10^6 cells/ml should also be tested. For adherent cells, 60~70% confluency at the time of transfection is optimal for most of transfections.