

Technical Note & Transfection Tips

----- Guidelines for Successful Plasmid DNA Transfection using Trans-Hi™

General Good DNA Transfection Practices

- Use high quality plasmid purification kits to obtain endotoxin free high purity DNA without RNA or protein contamination for higher transfection efficiency and improved reproducibility. Determine the DNA purity by measuring the OD 260/280 ratio, which should be between 1.7~1.9. Higher or lower ratios indicate impurities and should not be used in transfection experiments
- Use a positive control such as GFP or LacZ reporter plasmid to assess transfection efficiency and optimize transfection conditions.
- Use healthy cells. Passage cells at least twice after thawing to allow recovery before transfection, and use cells at low passage number (<16 passages). Discard cells if they have become overconfluent. Regularly check for contaminants: yeast, bacteria and mycoplasma.
- For long term storage, keep Trans-Hi™ Transfection Reagent at 4°C and do not freeze.

Transfection Tips

- The day before transfection, seed the cells to obtain ~80% confluency at the time of transfection. Perform transfection 1 day after cell seeding. If plated >1 day prior to transfection, the transfection efficiency may decrease.
- The optimal ratio of Trans-Hi™/DNA ranges from 2:1 to 3:1. For initial transfection, we recommend the ratio of Trans-Hi™/DNA at 3:1 and optimize if necessary.
- Prior to transfection, make Trans-Hi™/DNA mix using serum-free DMEM. **Note:** Never use Opti-MEM to dilute Trans-Hi™ reagent and plasmid. The trace of serum from Opti-MEM may interfere the formation of Trans-Hi™/DNA complex.
- The optimal time and temperature for the formation of Trans-Hi™/DNA transfection complex is ~10 minutes at room temperature (RT). Note: Never leave the transfection complex at RT longer than 20 minutes before addition to mammalian cell culture.
- Please try the General Transfection Protocol first. Give Advanced Transfection Protocol a try only when you are NOT satisfied with transfection efficiency of the general protocol.

Tips to Increase DNA Transfection Efficiency

- Increase DNA amount up to 2 folds while locking the ratio of Trans-Hi™/DNA.
- Test Trans-Hi™/DNA ratio ranging from 2:1 to 3:1.
- Just after transfection, centrifuge the plates 3 min at 200 g.

Tips to Increase Cell Viability

- Replace medium after 5~6 hours after transfection.
- Decrease DNA amount by half or more while locking the ratio of Trans-Hi™/DNA.
- Perform transfection at higher cell confluency such as 90%.
- Check that the target gene does not affect cell viability.