MATra Troubleshooting

Low Transfection Efficiency

- **Inappropriate buffer composition:**
  Avoid serum or other charged macromolecules when incubating magnetic particles and nucleic acids, otherwise proteins from serum will bind to the MATra-A Reagent; once the MATra-A/DNA complex is formed it can be applied to cells in the presence of serum.

- **Suboptimal ratio of MATra-A Reagent to nucleic acid or virus:**
  Optimize/titrate amount of nucleic acid:bead complex to be applied to the cells by using the titration protocol in the 96 well format (see manual).

- **Optimize cell density**

- **Extend readout time**
  e.g. 24 h longer than usual.

- **Incubation time:**
  Incubate cells for prolonged time in the magnetic field.

- **Cell condition:**
  Use freshly thawed cells that have been passaged at least once. Cells that have been in culture for a long time may become resistant to transfection.

- **Use other cell line if possible**

- **Positive control:**
  Perform a positive control transfection experiment with a well-characterized reporter gene (e.g. GFP, Luciferase).

- **Mycoplasma contamination:**
  Mycoplasma contamination alters transfection efficiency.

Inhomogeneous Transfection

- **Inhomogeneous mixture of nucleic acid:bead complex and cell culture:**
  Mix the nucleic acid:MATra bead complex and the cell culturing medium supernatant over the cells thoroughly prior to placing the culturing plate onto the magnet plate. Do not mix by circular motion. It may be recommendable, particularly in case of large surfaces, to add the culture supernatant to the

formed bead:nucleic acid complex in a suitable vessel, to mix thoroughly and then to apply the homogenous mixture back to the cells.

- **Reduce time of complex formation between the magnetic beads and nucleic acids**

Cellular toxicity

- **Cell density (% confluence) was not optimal at the time of transfection:**
  Adherent cells are seeded such that they reach 30-60% density at the time of MATra. If the cell density is too low, increased toxicity may be observed. For suspension cells it is necessary that the cells are immobilized on the well bottom (see manual).

- **Incubation time after transfection:**
  Reduce the incubation time of complexes with the cell. Medium can be changed 4 – 6 hours after performing MATra (depending on the cell type).

- **Suboptimal amount of nucleic acid or transfection reagent (see manual):**
  Reduce amount of nucleic acid:bead complex to be applied to the cells. Higher amounts of MATra-A/MATra-si Reagent or MA Lipofection Enhancer may be toxic for cells and can reduce transfection efficiency. For MATra approximately 5 x less DNA compared to lipofection is necessary.

- **Purity of transfecting molecule:**
  Check the purity of the molecule of interest to be delivered, e.g. lipopolysaccharides which are endotoxins will cause cell death.

- **Use other cell line if possible**