

# Guidelines for successful plasmid DNA transfection using jetPRIME®

## Good DNA transfection practices

- Use a reporter gene to set up and optimize transfection conditions, as those may vary depending on the cells to transfect. Various reporter systems are commercially available: Renilla/Luciferase and GFP (Green Fluorescent Protein) are the most commonly used.
- Use high quality plasmid purification kits to obtain high grade DNA, without RNA or protein, for higher transfection efficiency and improved reproducibility.
- Passage cells at least twice after thawing to allow recovery before transfection and use cells at low passage number (< 20 passages). Discard cells if they have become overconfluent. Regularly check for contaminants: yeast, bacteria and mycoplasma.
- Check transfection efficiency before purchasing a new batch of serum or trypsin.
- Store appropriately jetPRIME® (4°C) and DNA.

## Prepare the plasmid DNA

- Measure UV absorbance at 280 nm. OD260/280 ratio should be approximately 1.8.
- Resuspend the plasmid in deionized water or TE buffer at a concentration of ca. 1 µg/µl.
- Aliquot the plasmid preparation and store it at -20°C.
- Check for RNA contamination by agarose gel electrophoresis and ethidium bromide staining.

## Transfection tips

- The day before transfection, seed the cells to obtain 60-80% confluency at the time of transfection.
- Prior to transfection, dilute the DNA in the provided jetPRIME® buffer first, and then add the jetPRIME® reagent.

## Tips to increase DNA transfection efficiency

- Increase DNA amount up to 2-fold.
- Test higher DNA/jetPRIME® ratios such as 1:3 or 1:4.
- Just after transfection, centrifuge the plates 5 min at 180 g.

## Tips to increase cell viability

- Replace medium after 4 hours.
- Decrease DNA amount by half or more.
- Analyze transfection at an earlier time point (24 h after transfection instead of 48 h, for instance).
- Verify that the expressed protein does not affect cell viability.

## DNA transfection protocol using jetPRIME® in 6-well plates

