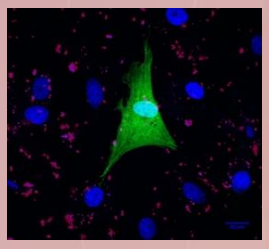
Tailored transfection reagent: *Prime-Fect*

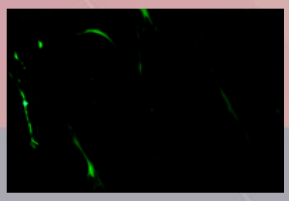
PRODUCT NUMBERS: 20-10 and 20-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: -20 °C

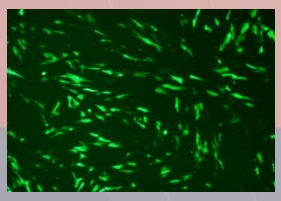
Product Description

Prime-Fect is a highly effective transfection reagent for attachment-dependent primary cells. **Prime-Fect** is a synthetic amphiphilic polymer that is tailored primarily for DNA delivery to a wide range of primary cells. It is capable of undergoing multivalent interactions with polynucleotides and encapsulating co-incubated polynucleotides into 100-200 nm particles with a net positive charge. The complexation between the polynucleotides and the **Prime-Fect** occurs in aqueous buffers, obviating the need for organic solvents during preparation. **Prime-Fect** is a non-integrating carrier, so that the genetic make-up of host cells is not altered after treatment with the transfection reagent. **Prime-Fect** has been tested and also found effective for siRNA delivery to different types of attachment dependent cells. As with all transfection reagents, formulations of **Prime-Fect** with DNA or RNA may need to be optimized for specific cell types and transfection conditions.

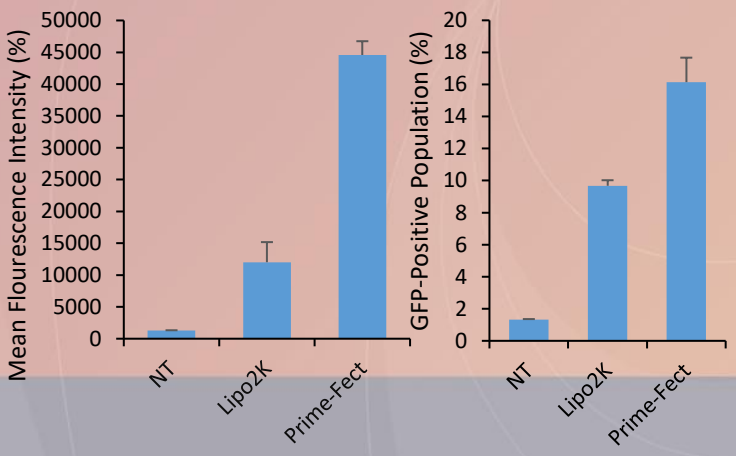
Transfecting bone marrow stromal cells with Prime-Fect. GFP expression was induced with a plasmid and analyzed by fluorescent microscopy 2 days after transfection. Cell-associated DNA is visualized with a red label.







Transfecting umbilical cord-derived mesenchymal stem cells with Prime-Fect. GFP expression was induced with a plasmid DNA and analyzed by fluorescent microscopy 2 days after transfection. (**Left**) A leading polymeric transfection reagent and (**right**) Prime-Fect.



Transfecting human bone marrow stromal cells with DNA using Prime-Fect. GFP expression was induced with a plasmid and analyzed by flow cytometry 2 days after expression. Results are displayed comparing Prime-Fect to a leading lipofection reagent with Mean Fluorescence Intensity (Left), and % GFP-Positive Population (Right).

| Benefits of Prime-Fect

- High transfection efficiency in the presence of serum.
- Effective delivery of DNA or RNA reagents via a simple protocol.
- Minimal toxicity compared to other commercial reagents, minimally affecting highly sensitive primary cells.
- Non-integrating transfection reagent eliminates adverse effects due to host genome integration.

| Transfection Protocol

The following procedure is recommended for preparation of plasmid DNA or siRNA particles with **Prime-Fect** and subsequent transfection of attached cells. Please ensure all reagents are at room temperature for the procedures.

- We recommend using 30-50% confluent cells for transfection. Cells can be seeded at desired concentrations in multiwell plates 24 hours before the incubation with complexes.
- Recommended amounts of DNA/siRNA and **Prime-Fect** reagent are shown in the Table for different multiwell plates. The final recommendations for plasmid DNA and siRNA are 0.5-1.5 µg/mL. For **Prime-Fect**, we recommend a final concentration of 2.5 to 15.0 µg/mL, with typical nucleic acid: **Prime-Fect** ratios of 1:5 to 1:10.
- We recommend all concentrations and reagent ratios to be optimized in the hands of practitioners. The amounts shown below are for a single well, assuming 0.1 mg/mL plasmid DNA and 1 mg/mL **Prime-Fect**

stock solutions. Once the plate format is selected, complex volumes should be adjusted based on number of replicates.

- We recommend preparing of 10% excess volume to account for any possible loss due to pipetting.
- DMEM (or equivalent) medium without antibiotics or serum is recommended for complex preparation but the medium can be changed depending on the need of the cells.

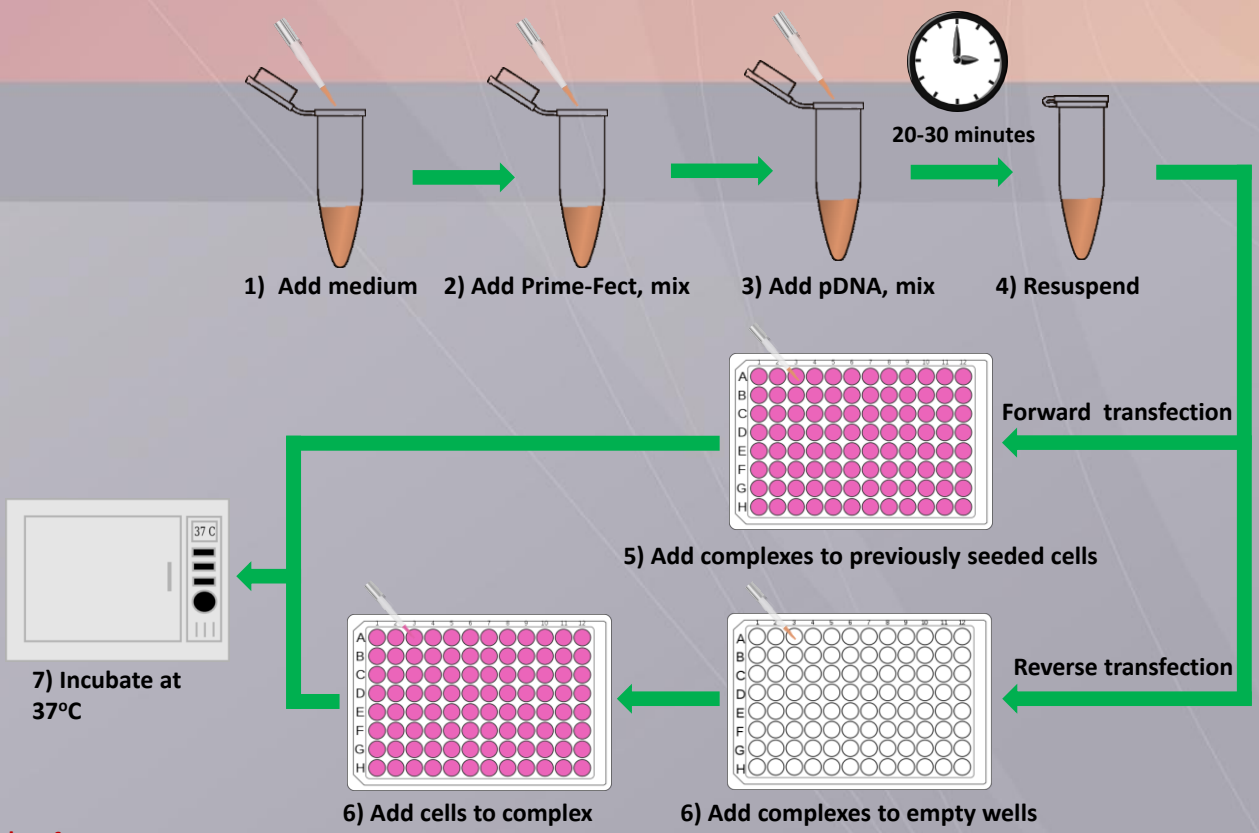
Plate Format	Medium (µL)	pDNA (µL) *	Prime-Fect (µL)	Total Complexes (µL)	Culture Volume (µL)
96-well	7.25	1.0	0.75	10.0	90.0
48-well	11.75	3.0	2.25	20.0	280.0
24-well	33.5	6.0	4.5	50.0	550.0
6-well	217.5	30.0	22.5	300.0	2700.0

Recommended volumes for 0.1 mg/mL DNA and 1 mg/mL **Prime-Fect** solutions. Final DNA: **Prime-Fect** ratio is 1:7.5.

| Procedure

1. Add the desired volume of medium to 1.5 mL Eppendorf microcentrifuge tubes.
2. Add undiluted **Prime-Fect** solution to the medium in tubes (Step #1) and vortex gently for 3 sec.
3. Add pDNA to the polymer solution. Vortex for 3 sec and incubate for 30 min.
4. Re-suspend the pDNA complexes in solution using a pipette at the end of the incubation.
5. **Forward transfection**: add complexes directly to the wells containing the previously seeded cells (allowed to attach for 24 hours in complete medium with serum). Ensure even distribution, gently shake plates if necessary.
6. **Reverse transfection**: add complexes to empty wells, followed by the addition of cells suspended in complete medium with serum. Gently shake the plate to ensure uniform distribution of cells in wells.
7. Incubate the plate under cell culture conditions for culture. Sample cells at desired times for analysis.

| Graphical Procedure for complex preparation and transfection



| References

- KC et al., J. Materials Chemistry B (2015) 3: 3972-3982