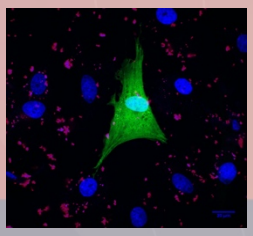
tailored transfection reagent: Prime-Fect

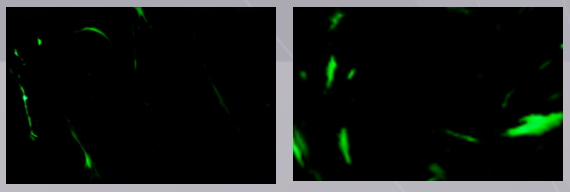
PRODUCT NUMBERS: 20-10 and 20-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: 4 °C

Product Description

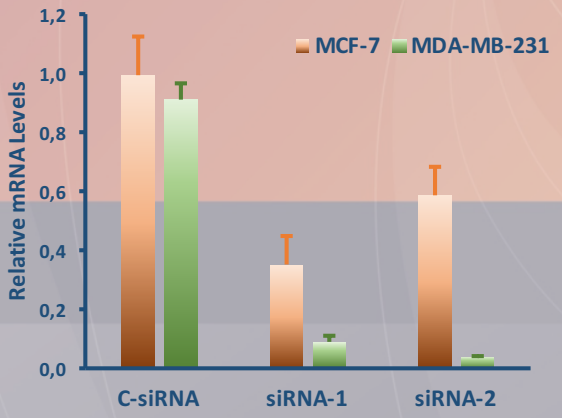
Prime-Fect is a highly effective transfection reagent for attachment-dependent primary cells. **Prime-Fect** is a synthetic amphiphilic polymer that is tailored primarily for DNA delivery to a wide range of primary cells. It is capable of undergoing multivalent interactions with polynucleotides and encapsulating 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 breast cancer MDA-MB-231 and MCF-7 cells with siRNA using Prime-Fect. The expression levels of two target genes were analyzed by qPCR after delivery of control (scrambled) and gene-specific siRNAs (2 days after transfection).

| 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 to use 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 below for different multiwell plates. The final recommendations for plasmid DNA and siRNA are 0.5-1.5 µg/mL and 30 to 60 nM, respectively. For **Prime-Fect**, we recommend a final concentration of less than 10 µg/mL, with typical nucleic acid:**Prime-Fect** ratios of 1:5. 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 0.4 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 preparation 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)	DNA (µL)*	Prime-Fect (µL)*	Medium Volume (µL)
96-well	10	0.25	0.5	90
48-well	30	0.75	1.5	270
24-well	60	1.5	3.0	540
6-well	300	7.5	15.0	2700

Recommended volumes for 0.4 mg/mL DNA and 1 mg/mL **Prime-Fect** solutions. Final DNA:**Prime-Fect** ratio is 1:5.

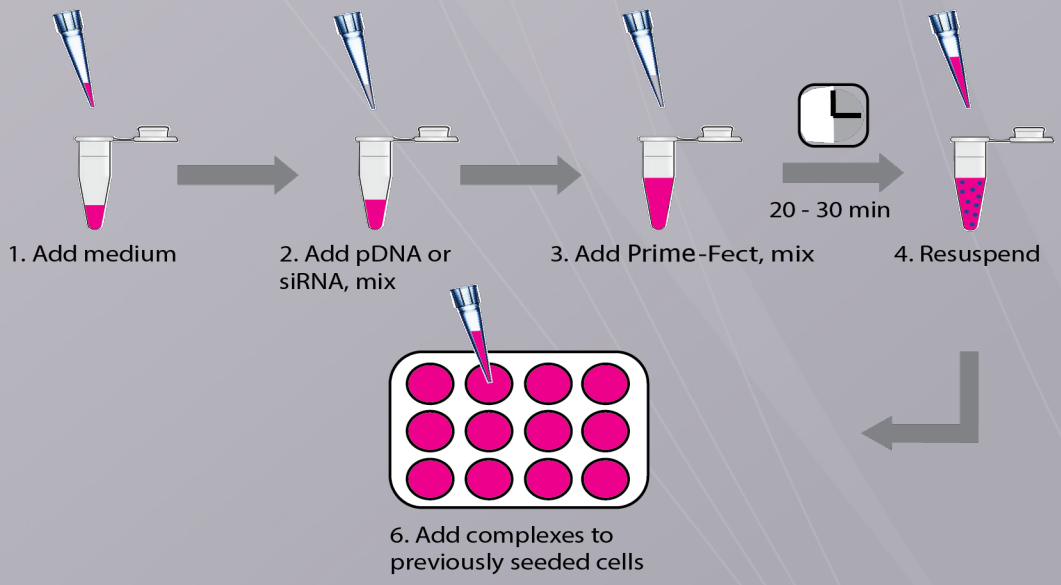
Plate Format	Medium (µL)	siRNA (µL)*	Prime-Fect (µL)*	Medium Volume (µL)
96-well	10	0.3	0.2	90
48-well	30	0.9	0.6	270
24-well	60	1.8	1.3	540
6-well	300	9.0	6.3	2700

Recommended volumes for 10 µM siRNA and 1 mg/mL **Prime-Fect** solutions. Final siRNA:**Prime-Fect** ratio is 1:5.

| Procedure (described for plasmid DNA; siRNA can be substituted for DNA in case of siRNA transfections)

1. Add desired volume of medium to 1.5 mL plastic (microcentrifuge) tubes.
2. Add appropriate volume of DNA (siRNA) solution to the medium in tubes and vortex gently for 3 sec.
3. Add undiluted **Prime-Fect** solution to nucleic acid solution. Vortex for 3 sec and incubate for 20-30 min.
4. Re-suspend the complexes in solution using a pipette at the end of the incubation.
5. Add complexes to the wells containing cells and ensure even distribution – gently shake plates if necessary.
6. Incubate the plate under conditions suitable for cell culture and sample cells at desired times for analysis.

| Graphical Procedure



| References

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