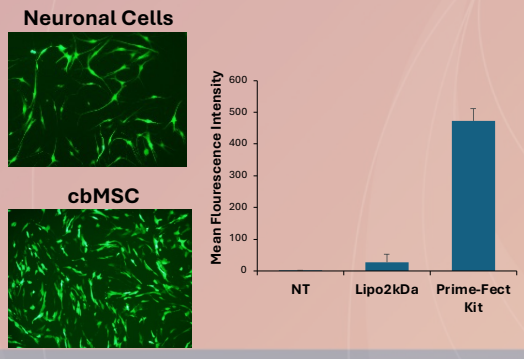
Tailored Transfection Reagent: *Prime-Fect Kit*

PRODUCT NUMBERS: 20-40 and 20-50	SIZE: 0.75 and 1.5 mL	STORAGE: -20 °C	CONCENTRATION: 1.0 mg/mL (Kit A), 0.4mg/mL (Kit B)

Product Description

The **Prime-Fect Kit** (Component A and Component B) is a highly effective transfection reagent combination optimized for attachment-dependent cells. The kit components are derived from synthetic amphiphilic polymers tailored for pDNA and siRNA delivery after extensive testing of polymer libraries. It can undergo multivalent interactions with nucleic acids and encapsulating them into nanoparticles (~100 nm) appropriate for effective cellular uptake. This interaction occurs in aqueous buffer, obviating the need for organic solvents during preparation. The **Prime-Fect Kit** components are non-integrating carriers, so that the genetic make-up of host cells is not altered after the treatment. These materials have been tested and found effective in primary cells and in certain cell lines. As with all transfection reagents, formulations of **Prime-Fect Kit** with polynucleotides may need to be optimized for specific cell types and transfection conditions.

Transfecting neuronal cells (dorsal root ganglion from mice) and human cord-blood derived mesenchymal stem cells (cbMSC) with Prime-Fect Kit. A pDNA coding for GFP protein were used to express GFP in primary cells. **Left**. Fluorescence microscopy analysis of the transfected cells. [pDNA] = 1 mg/mL. pDNA:Prime-Fect (A):Trans-Booster (B) ratios of 1:5:1. **Right**. Flow cytometric analysis of GFP expression in cbMSC. The extent of GFP expression was shown as mean fluorescence intensity (MFI). Lipofectamine 2000 was used as a reference transfection reagent. NT: Not treated.



| Benefits of Prime-Fect Kit

- High transfection efficiency in the presence of serum.
- Effective delivery of nucleic acids via a simple protocol that is ideal for scale-up, automation and optimization.
- Minimal toxicity compared to commercial reagents, suitable for highly sensitive human cells.
- Non-integrating transfection reagent eliminates adverse effects due to host genome integration.
- Possible to use the same transfection reagent in animal models, leading to harmonized studies.
- Flexibility to optimize the formulation for particular cell types/nucleic acids due to 2 component system

| Notes on Transfection Protocol

The following procedure is recommended for preparation of siRNA complexes with Prime-Fect Kit, and subsequent transfection. Please ensure all reagents are at room temperature for the procedures.

- Cells can be seeded at desired concentrations in multiwell plates. We recommend using freshly passaged cells (P4 to P20) at exponential growth phase for transfection.
- For Attachment dependent cells: allow 24 hours for cells to attach and spread. For suspension growing cells: complexes could be added in multiwell plates first, followed by the addition of cells.
- The final recommended concentration for siRNA: 40 to 60 nM, Prime-Fect Kit A: 2.8 to 8.4 µg/mL and Prime-Fect Kit B: 0.56 to 0.84 µg/mL.
- The recommended ratio of nucleic acid: **Prime-Fect Kit A** component is 1:5 to 1:10. And the recommended ratio of nucleic acid: **Prime-Fect Kit B** component is 1:0.5 to 1:1.
- We recommend all concentrations and reagent ratios to be optimized for each cell type and nucleic acid. The amounts shown below are for a single well, assuming 0.14 µg/mL siRNA solution, 1 mg/mL **Prime-Fect Kit A** solution **(as supplied)** and 0.2 mg/mL **Prime-Fect Kit B** solution (diluted x2 from supplied solution). Once the plate format is selected, complex volumes should be adjusted based on the number of replicates.

Plate Format	Medium (µL)	siRNA (µL) *	Prime-Fect Kit B (µL)	Prime-Fect Kit A (µL)	Culture Volume per well (µL)
96-well	8.35	0.6	0.42	0.63	90
48-well	15.05	1.8	1.26	1.89	270
24-well	40.10	3.6	2.52	3.78	540
6-well	250.50	18.0	12.6	18.90	2320

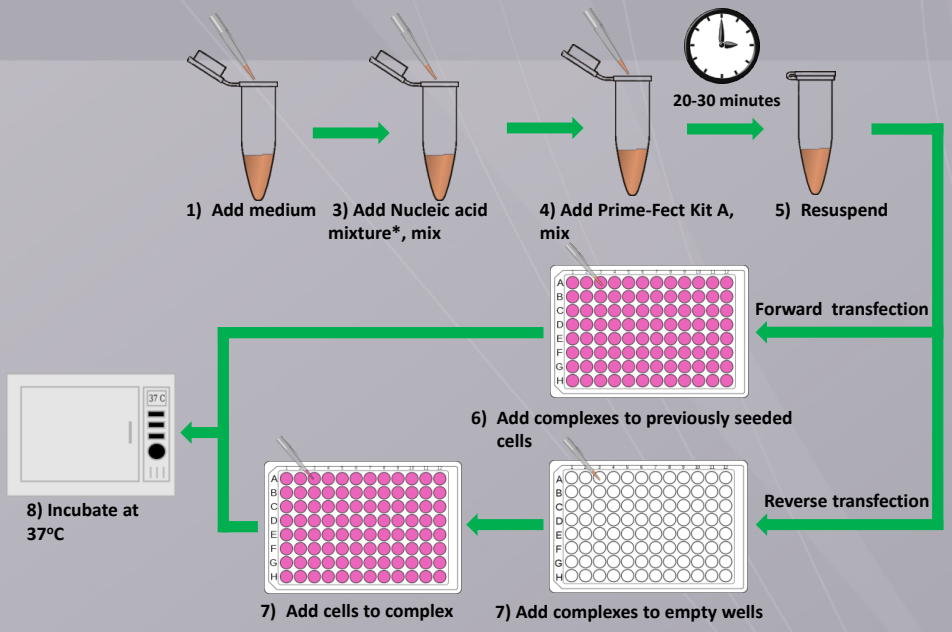
* Recommended volumes to make transfection complexes if using 0.14 µg/mL siRNA solution, 1 mg/mL **Prime-Fect Kit A** solution and 0.2 mg/mL **Prime-Fect Kit B** solution. These ratios give pDNA:**Prime-Fect Kit A** ratio of 1:7.5 and pDNA: **Prime-Fect Kit B** ratio of 1:1.

- We recommend preparation of 10% excess volume to account for any pipetting losses.
- DMEM (or equivalent) cell culture medium without antibiotics or serum is recommended for complex preparation, but the medium can be changed depending on the need of the cells.
- The Table above can be adopted for preparation of DNA complexes. We recommend DNA: **Prime-Fect Kit A** ratio of 1:5 or 1:10 w/w, and DNA: **Prime-Fect Kit B** ratio of 1:1 w/w.

| Step-by-Step Procedure

1. Add an appropriate volume of medium into 1.5 mL Eppendorf tubes.
2. Separately, prepare nucleic acid mixture by adding desired volume of siRNA solution and Prime-Fect Kit B.
3. Add the nucleic acid mixture to the Eppendorf tube above and vortex gently for 3 sec.
4. Add **Prime-Fect Kit A** solution to the nucleic acid solution above. Vortex for 3 sec and incubate for 20-30 min.
5. Re-suspend the complexes in solution using a pipette at the end of the incubation period.
6. **Forward transfections**: add complexes to 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.
7. **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.
8. Incubate the plate under cell culture conditions for culture. Sample cells at desired times for analysis.

| Graphical Procedure for Preparation of Transfection Complexes and Treatment



2) * Nucleic acid mixture: Separately prepare nucleic acid mixture by adding desired volume of siRNA solution and Prime--Fect Kit B and follow the step 3.