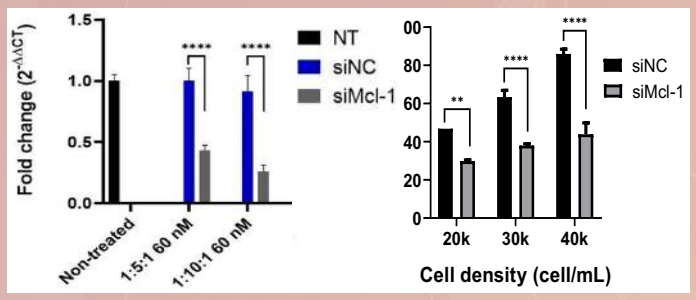
Tailored Transfection Reagent: Prime-Fect (Kit)

PRODUCT NUMBERS	SIZE	STORAGE	CONCENTRATIONS
20-40 and 20-50	0.75 and 1.5 mL	-20 °C	1 mg/mL (A) and 0.4 mg/mL (B)

Product Description

The **Prime-Fect Kit** (Reagent A and 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 MDA-MB-436 cells with Prime-Fect Kit. An siRNA targeting anti-apoptotic protein Mcl-1 (siMcl-1) and a scrambled control siRNA (siNC) were used to silence Mcl-1 and inhibit cell growth. **Left**. PCR assessment of target (Mcl-1) mRNA levels in treated cells. [siRNA] = 60 nM. siRNA:Prime-Fect (A):Trans-Booster (B) ratios of 1:5:1 and 1:10:1. **Right**. Cells were seeded at 20, 30 and 40K/mL and cell viability was determined by the MTT assay after 3 days of treatment.

| 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 pDNA complexes with **Prime-Fect Kit**, and subsequent transfection of cells. Please ensure all reagents are at room temperature for the procedures.

- We recommend using freshly passaged cells (P4 to P20) at exponential growth phase for transfection.
- Cells can be seeded at desired concentrations in multiwell plates before addition of complexes (normal transfection). If cells are attachment-dependent, allow 24 hours for attachment. For suspension-growing cells, complexes can be incubated in multiwell plates first, followed by addition of desired cells (reverse transfection).
- Recommended amounts of pDNA and **Prime-Fect Kit** are shown in the Table below for different multiwell plates. The final recommendations for pDNA concentration are 0.25-1.0 µg/mL, for **Prime-Fect Kit A** component are 1.25 to 5 µg/mL and for **Prime-Fect Kit B** component are 0.25-1.0 µg/mL.
- The recommended ratio of nucleic acid:**Prime-Fect Kit A** component is 1:5 to 1:10.
- 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 0.2 mg/mL pDNA solution, 1 mg/mL **Prime-Fect A** solution **(as supplied)** and 0.2 mg/mL **Prime-Fect 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)	pDNA (µL) *	Prime-Fect Kit B (µL)	mRNA-Fect A (µL)	Culture Volume per well (µL)
96-well	9	0.75	0.75	1.5	90
48-well	24	1.5	1.5	3.0	270
24-well	48	3.0	3.0	6.0	540
6-well	144	9.0	9.0	18.0	2320

* Recommended volumes to make transfection complexes if using 0.2 mg/mL pDNA, 1 mg/mL **Prime-Fect Kit A** and 0.2 mg/mL **mRNA-Fect Kit B**. These ratios give pDNA:**Prime-Fect Kit A** ratio of 1:10 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 siRNA complexes. We recommend siRNA:**Prime-Fect Kit A** ratio of 1:5 or 1:10 w/w, and siRNA:**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. Add the desired volume of pDNA solution and **Prime-Fect Kit B** component to the Eppendorf tube above and vortex gently for 3 sec.
3. Add **Prime-Fect Kit A** solution to the nucleic acid solution above. 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 period.
5. For normal transfection, 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.
6. For 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 preparation of transfection complexes and treatment