Tailored Transfection Reagent: *ALL-Fect Kit*

PRODUCT NUMBERS: 10-40 and 10-50	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 **ALL-Fect Kit** (components A and B) is a highly effective transfection reagent combination optimized for DNA/RNA delivery to both attachment-dependent and suspension-growing cells. The kit components are based on synthetic cationic lipopolymer polymers tailored primarily for DNA delivery after extensive testing of polymer libraries. The components can undergo multivalent interactions with mRNA, encapsulating it into polyionic nanoparticles (~100 nm) appropriate for effective cellular uptake. This interaction occurs in aqueous buffer, obviating the need for organic solvents during complex preparation. The **ALL-Fect Kit** components are non-integrating carriers, so that the genetic make-up of host cells is not altered after the treatment. These transfection reagents have been tested and found effective for plasmid DNA and mRNA delivery to a wide range of cell types. As with all transfection reagents, formulations of **ALL-Fect Kit** with polynucleotides may need to be optimized for specific applications and transfection conditions.

Transfecting Jurkat cells with All-Fect Kit.
A pDNA coding for a reporter protein (Green Fluorescent Protein, GFP) was used to assess the efficiency of pDNA expression. Typical GFP expression levels were visualized under fluorescent microscopy. For comparison, a leading lipofection reagent was used according to the manufacturer's instructions.

| Benefits of ALL-Fect Kit

- High transfection efficiency in the presence of serum.
- Effective delivery of mRNAs 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 All-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 recommendations for pDNA concentration are 0.25-1.0 µg/mL, for All-Fect Kit A component are 1.25 to 10 µg/mL and for All-Fect Kit B component are 0.25-1.0 µg/mL.
- The recommended ratio of nucleic acid: All-Fect Kit A component is 1:5 to 1:10 and the recommended ratio of nucleic acid: All-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.
- Recommended amounts of DNA and ALL-Fect Kit shown in the Table below for different multiwell plates. The amounts are for a single well, assuming 0.1 mg/mL pDNA solution, 1 mg/mL All-Fect A solution and 0.1 mg/mL ALL-Fect B solution. Once the plate format is selected, complex volumes should be adjusted based on the number of replicates.

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

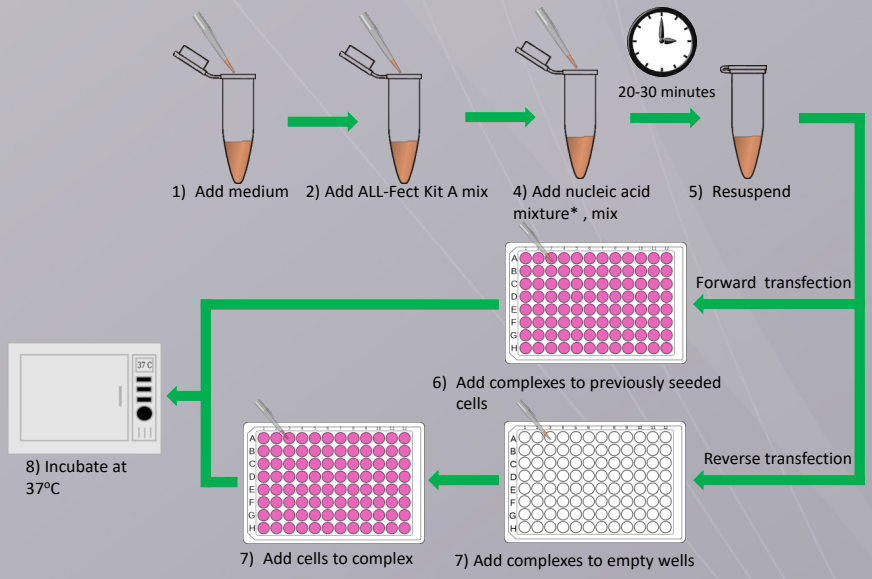
*Recommended volumes to make transfection complexes if using 0.1 mg/mL pDNA, 1 mg/mL All-Fect Kit A and 0.1 mg/mL All-Fect Kit B. These ratios give pDNA: ALL-Fect Kit A ratio of 1:7.5 and pDNA: All-Fect Kit B ratio of 1:1. It is recommended to mix DNA and ALL-Fect Kit B in a separate viral before adding to the media for complexation.

- Different buffers can be used to make up the complexes for administration. Phosphate Buffer Saline (PBS), Hank's Balanced Salt Solution (HBSS; Ca^{+2}/Mg^{+2} free) or tissue culture medium (e.g., DMEM, RPMI) without serum/antibodies can be used for making the complexes and injection.
- We recommend preparation of 10% excess volume to account for any pipetting losses.
- We recommend all amounts and reagent ratios to be optimized for particular applications. The amounts below are for a single injection with a volume of 100 µL. The volume can be adjusted depending on the number of replicates.

| Step-by-Step Procedure

1. Add an appropriate volume of medium into 1.5 mL Eppendorf tubes.
2. Add ALL-Fect Kit A solution to the media step #1.
3. Separately, prepare nucleic acid mixture by adding desired volume of DNA solution and ALL-Fect Kit B.
4. add directly to the polymer solution of step #2. vortex gently 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 Complexes Preparation and Transfection



3) * Nucleic acid mixture: Separately prepare nucleic acid mixture by adding desire volume of DNA solution to ALL-Fect Kit B and follow the step 4