Tailored Transfection Reagent: *ALL-Fect*

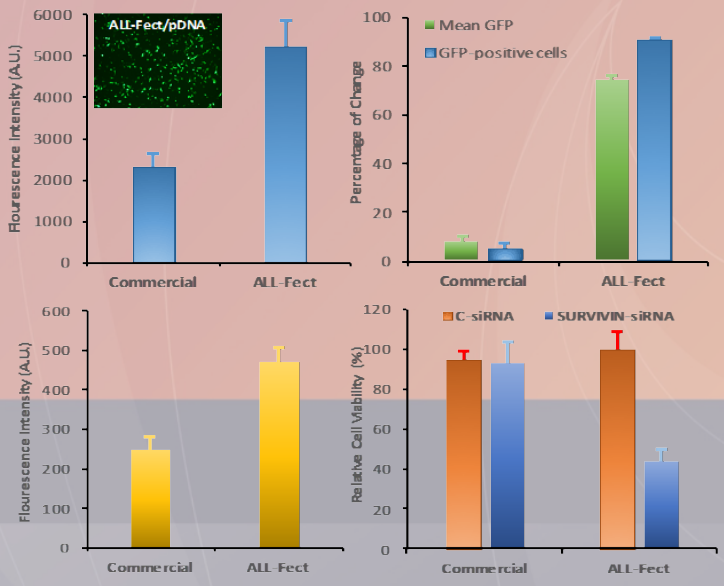
PRODUCT NUMBERS: 10-10 and 10-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: -20 °C

Product Description

ALL-Fect is a highly effective, broadly acting transfection reagent for attachment dependent and independent cells. **ALL-Fect** is a synthetic cationic lipopolymer that is tailored for DNA, mRNA and siRNA delivery to a wide range of cells. It is capable of undergoing multivalent interactions with polynucleotides and encapsulating co-incubated polynucleotides into polyionic nanoparticles of size 100-200 nm. The complexation between the polynucleotides and the **ALL-Fect** occurs in aqueous buffers, obviating the need for organic solvents during preparation. **ALL-Fect** is a non-integrating carrier, so that the genetic make-up of host cells is not altered after treatment with the transfection reagent. **ALL-Fect** has been tested and found effective in different types of attachment dependent cells as well as suspension-growing cells. As with all transfection reagents, formulations of **ALL-Fect** with DNA or RNA may need to be optimized for specific cell types and transfection conditions.

Transfecting attachment-dependent MDA-MB-231 cells with plasmid DNA and siRNA using ALL-Fect and a leading lipofection reagent. (Left) GFP expression was induced with plasmid DNA and analyzed by fluorescent microscopy (insert) and flow cytometry 2 days after transfection. (**Right**) GFP silencing was induced with siRNA and analyzed by flow cytometry 2 days after transfection.

Transfecting attachment-independent K562 cells with plasmid DNA and siRNA using ALL-Fect and a leading lipofection reagent. (Left) GFP expression was induced with plasmid DNA and analyzed by flow cytometry 2 days after transfection. (**Right**) Survivin silencing and resultant inhibition of cell growth with specific siRNA 3 days after transfection.



| Benefits of ALL-Fect

- High transfection efficiency for both DNA and RNA reagents in the presence of serum.
- Effective delivery of both DNA and RNA reagents simultaneously via a simple protocol.
- Minimal toxicity compared to other commercial reagents, leading to more physiological responses.
- Non-integrating transfection reagent eliminates adverse effects due to host genome integration.
- Transfection reagent suitable for both attachment-dependent and attachment-independent cells

| Transfection Protocol

The following procedure is recommended for preparation of plasmid DNA (pDNA) particles with **ALL-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

- **pDNA Transfection**. Recommended amounts of pDNA and **ALL-Fect** reagent are shown in the Table below for different multiwell plates. The final recommended concentration for pDNA is 0.5 to 1.0 µg/mL.
- **ALL-Fect**, we recommend using nucleic acid: **ALL-Fect** ratios of 1:5 to 1:10. We recommend all concentrations and reagent ratios to be optimized for specific cell types. The amounts shown below are for a single well, assuming 0.4 mg/mL DNA and 1 mg/mL **ALL-Fect** stock solutions. Once the plate format is selected, complex volumes should be adjusted based on the number of replicates.
- We recommend using ALL-Fect Kit supplied for better transfection in **hard-to-transfect cells.**

- 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. For example, RPMI1640 can be used instead of DMEM

Plate Format	Medium (µL)	pDNA (µL) *	ALL-Fect (µL)	Total Complexes (µL)	Culture Volume (µL)
96-well	9.0	0.25	0.75	10.0	90.0
48-well	17.0	0.75	2.25	20.0	280.0
24-well	44.0	1.5	4.5	50.0	550.0
6-well	270.0	7.5	22.5	300.0	2700.0

*Recommended volumes for 0.4 mg/mL DNA and 1 mg/mL **ALL-Fect** solutions. Final DNA concentration is 1 µg/mL, and DNA: **ALL-Fect** ratio is 1:7.5.

| Procedure

1. Add the desired volume of medium to 1.5 mL Eppendorf microcentrifuge tubes.
2. Add undiluted **ALL-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