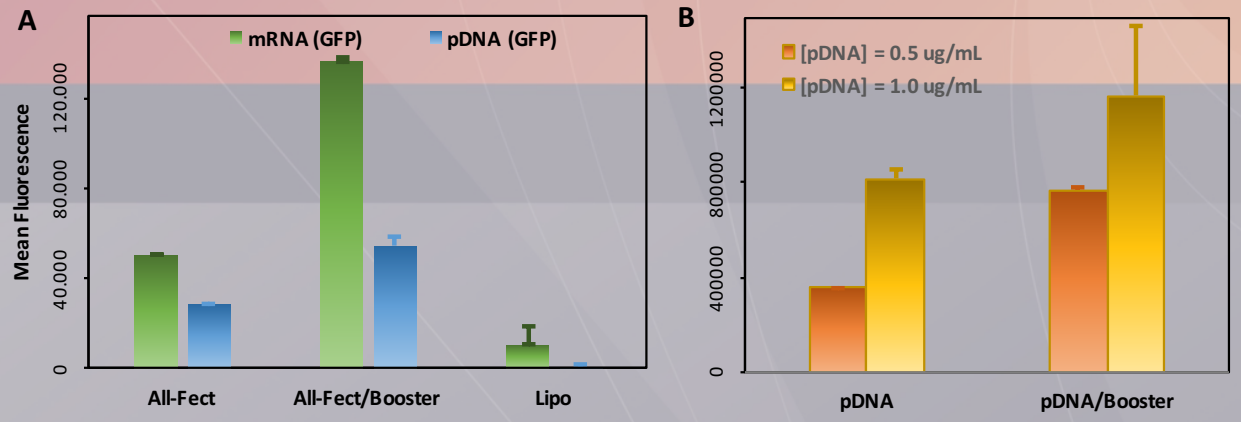
tailored transfection reagent: Trans-Booster

PRODUCT NUMBERS: 90-10 and 90-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 0.4 mg/mL	STORAGE: -20 °C

The ***Trans-Booster*** reagent is a supplement in usual transfection solutions that is optimized to enhance transfection efficiency of RJH Biosciences reagents. ***Trans-Booster*** reagent is a synthetic polymer that is tailored primarily for plasmid DNA (pDNA) and mRNA transfections. It is capable of undergoing multivalent interactions with polyplexes encapsulating pDNA and mRNA. Under some conditions, it was also found to be useful in transfection of smaller nucleic acids such as short interfering RNA (siRNA) and antisense oligonucleotides (ASO). The ***Trans-Booster*** reagents, as in all RJH Reagents, is formulated in an aqueous buffer, obviating the need for organic solvents during preparation. The ***Trans-Booster*** is a non-integrating reagent, so that the genetic make-up of host cells is not altered after treatment with this reagent. The ***Trans-Booster*** has been tested and found effective for pDNA and mRNA delivery in certain cell types and as with all transfection reagents, formulations of ***Trans-Booster*** may need to be optimized for specific cell types and transfection conditions.



Transfecting cells with the help of Trans-Booster. (A) Jurkat cells and (B) MDA-MB-231 cells transfected with All-Fect and All-Fect/Trans-Booster Combination. (A) An mRNA and a pDNA coding for a reporter protein (Green Fluorescent Protein, GFP) was used to assess the efficiency of GFP expression in Jurkat cells. The expression levels were quantitated by flow cytometry 48 hours after transfection and summarized as mean fluorescence (in au). For comparison, a leading lipofection reagent was used according to the manufacturer's instructions, but with and without Trans-Booster reagent. (B) pDNA coding for GFP was used to form complexes with All-Fect and All-Fect/Trans-Boost combination and exposed to MDA-MB-231 cells at 0.5 and 1.0 mg/mL concentrations. The expression levels were quantitated by flow cytometry 48 hours after transfection and summarized as mean fluorescence (in au).

| Notes on Transfection Protocol

The following procedure is recommended for preparation of pDNA particles with ***All-Fect*** and ***Trans-Booster***, and subsequent transfection of attachment-dependent and suspension-growing cells. Please ensure all reagents are at room temperature for the procedures. It is possible to use ***Trans-Booster*** with other RJH reagents.

- We recommend to use freshly passaged cells at exponential growth phase for transfection.

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- 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 cells to attach and spread. For suspension-growing cells, complexes could be incubated in multiwell plates first, followed by the addition of desired numbers of cells (reverse transfection).
- Recommended amounts of pDNA, **All-Fect** and **Trans-Booster** reagent are shown in the Table below for different multiwell plates. The final recommendations for pDNA are 0.5-1.0 µg/mL. We recommend a final concentration of 5-10 µg/mL for **All-Fect**, with typical nucleic acid:**All-Fect** ratio of 1:5 or 1:10. 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 pDNA, 0.4 mg/mL **Trans-Booster** and 1 mg/mL **All-Fect** solutions. Once the plate format is selected, complex volumes should be adjusted based on the number of replicates.
- We recommend preparation of 10% excess volume to account for any possible loss due to pipetting.
- 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.

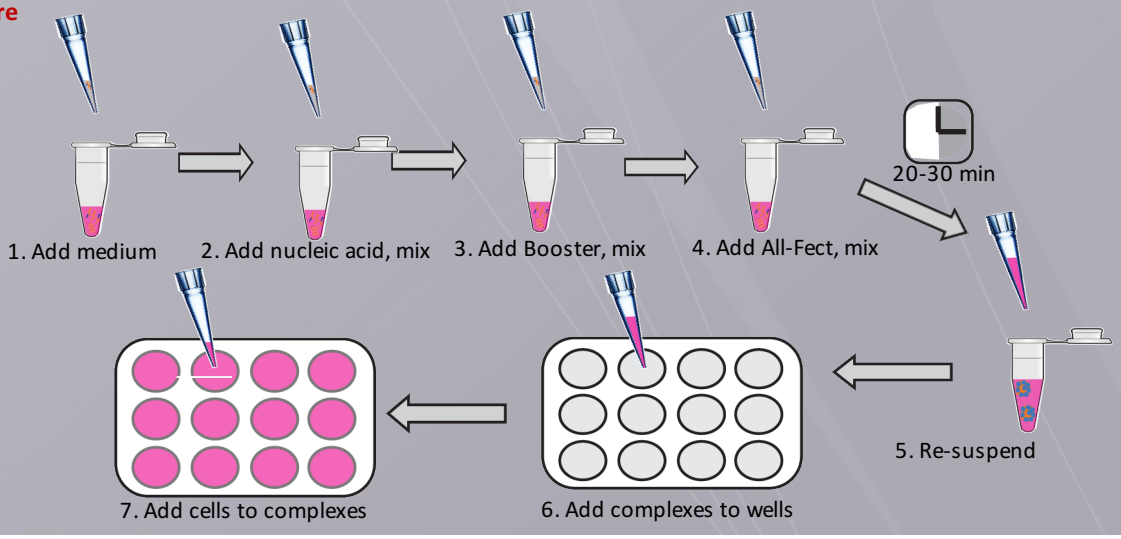
Plate Format	Medium (µL)	pDNA (µL)*	Trans-Booster (µL)*	All-Fect (µL)	Culture Volume (µL) per well
96-well	50	0.63	0.63	2.5	200
48-well	100	1.25	1.25	5	400
24-well	200	2.5	2.5	10	800
6-well	400	5	5	20	1600

* Recommended volumes for 0.4 mg/mL pDNA, 0.4 mg/mL **Trans-Booster** and 1 mg/mL **Prime-Fect** solutions (pDNA: **Prime-Fect** ratio is 1:10).

| Step-by-Step Procedure

1. Add desired volume of medium to 1.5 mL plastic (microcentrifuge) tubes.
2. Add appropriate volume of pDNA solution and **Trans-Boost** to the medium and vortex gently for 3 sec.
3. Add undiluted **All-Fect** solution to nucleic acid/**Trans-Booster** solution. Vortex gently for 3 sec and incubate for 20-30 min.
4. Re-suspend the complexes in solution using a pipette at the end of the initial 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 conditions suitable for cell culture (typically at 37 °C in a humidified atmosphere with 5% CO_2/95% air) and sample cells at desired times for analysis. We recommend 48 hours for analysis.

| Graphical Procedure



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