# *Trans*IT-PRO® Transfection Reagent for Protein Production in Expi293F™ Cells

Quick Reference Protocol

*Instructions for MIR 5720, 5730, 5740 and 5750*

## SPECIFICATIONS

| | |
|---|---|
| **Storage** | Store *Trans*IT-PRO® Reagent tightly capped at -20°C.<br>***Before each use***, warm to room temperature and vortex gently. |
| **Product Guarantee** | 1 year from the date of purchase, when properly stored and handled. |

## PLASMID DNA TRANSFECTION PROTOCOL

Full protocol and additional documentation available at ***mirusbio.com/5740***

**Fill in volumes below based on total culture volume (Table 1).**

**A. Maintenance of cells**

1. Passage Expi293F™ cells to a density of 2 x $10^6$ cells/ml in Expi293™ Expression Medium 18–24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection.
2. Culture cells overnight on an orbital shaker (125 rpm when using a shaker with a 2 cm orbital throw) at appropriate temperature and $CO_2$ levels (e.g. 37°C, 5-8% $CO_2$).

**B. Prepare *Trans*IT-PRO® Reagent:DNA complexes**

**NOTE:** This procedure recommends a 1:1 *Trans*IT-PRO® Reagent:DNA ratio, which is optimal for most proteins. However, doubling both DNA and reagent amounts (i.e. 60 µl *Trans*IT-PRO® Reagent + 60 µg total DNA per 30 ml culture volume) may result in improved expression with some constructs.

1. Determine cell density and viability. DO NOT proceed with transfection if cells are <95% viable.
2. Dilute Expi293F™ cells to a final density of 2.5 x $10^6$ cells/ml in Expi293™ Medium immediately before transfection. Return cells to the incubator while preparing transfection complexes.
3. Warm *Trans*IT-PRO® Reagent to room temperature and vortex gently.
4. Place ___ml of serum-free medium (e.g. Opti-MEM® or Opti-PRO™ SFM) in a sterile tube.
5. Add ___µg plasmid DNA. Mix gently by pipetting.
6. Add ___µl of *Trans*IT-PRO® Reagent. Mix gently by pipetting.
7. Incubate complexes at room temperature for 15-20 minutes.

**C. Distribute complexes to cells**

1. Add *Trans*IT-PRO® Reagent:DNA complexes to cultured Expi293F™ cells.
2. Culture transfected cells at appropriate conditions (e.g. 37°C, 5-8% $CO_2$, shaking) until harvest. The optimal incubation time for protein expression and harvest depends on the expression construct, nature of the protein, and detection method (i.e. 2-14 days post-transfection).
3. Harvest cells and/or supernatant and assay as required.

**Table 1**. Volume scaling worksheet for DNA transfections with *Trans*IT-PRO® Transfection Reagent.

| **Starting conditions per milliliter of complete growth medium** | | | | | |
|---|---|---|---|---|---|
| | Per 1 ml | | Total culture volume | | Reagent quantities |
| Serum-free Complex Medium | 0.1 ml | × | _____ml | = | _____ml |
| Plasmid DNA (1 µg/µl stock) | 1 µl | × | _____ml | = | _____µl |
| *Trans*IT-PRO® Transfection Reagent | 1 µl | × | _____ml | = | _____µl |

### Additional Information

Expi293™ and Expi293F™ are trademarks of Life Technologies Corporation.

*For additional optimization tips, see TransIT-PRO® Transfection Reagent full protocol.*

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## NOTES

**Reagent Agent®**

Reagent Agent® is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at: mirusbio.com/ra

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