*Trans*IT-PRO® Transfection Kit

Quick Reference Protocol

Instructions for MIR 5700 and 5760

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/5700

SPECIFICATIONS

Storage	Store both *Trans*IT-PRO® Reagent and PRO Boost Reagent tightly capped at -20°C. ***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/5700***

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

A. Maintenance of cells

1. Split suspension CHO or 293 cells 18–24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection.
2. Culture overnight.

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

1. Seed cells at a density of 2 x 10^6 cells/ml immediately before transfection.
2. Warm *Trans*IT-PRO® Reagent and PRO Boost Reagent to room temperature and vortex gently.
3. Place ___ml of serum-free medium (e.g. Opti-MEM® or OptiPRO™)* in a sterile tube.
4. Add ___µg plasmid DNA. Mix gently by pipetting.
5. Add ___µl of *Trans*IT-PRO® Reagent. Mix gently by pipetting.
6. **(Optional)** Add ___µl of PRO Boost Reagent. Mix gently by pipetting.
7. Incubate complexes at room temperature fo the recommended amount of time:
   For complexes that will be added to suspension 293 cells, incubate for 15-20 minutes.
   For complexes that will be added to suspension CHO cells, incubate for 5-10 minutes.

C. Distribute complexes to cells

1. Add *Trans*IT-PRO® Reagent:PRO Boost:DNA complexes to cultured cells.
2. Incubate cells for 2-14 days depending on cell type, culture temperature, nature of the protein, and detection method. For further optimization information, please see the full protocol.
3. Harvest cells and/or supernatant and assay as required.

*When using *Trans*IT-PRO® Reagent with the CHOgro® Expression System, form transfection complexes in CHOgro® Complex Formation Solution.

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

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® Reagent	1 µl	×	_____ml	=	_____µl
PRO Boost Reagent (optional)	0.5 µl	×	_____ml	=	_____µl

▸ Transfection Optimization

Determine the best *Trans*IT-PRO® Reagent:DNA ratio for each cell type. Start with 1 µl of *Trans*IT-PRO® Reagent per 1 µg of DNA. Vary the concentration of *Trans*IT-PRO® Reagent from 0.5–2 µl per 1 µg DNA to find the optimal ratio. Vary the concentration of Boost Reagent from 0–1.5 µl per 1 µg DNA. NOTE: The use of PRO Boost in transfections may enhance expression up to two-fold in certain CHO media formulations.

For additional optimization tips, see 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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