

TransIT-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 <i>TransIT-PRO</i> ® 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. Passage suspension CHO or 293 cells 18–24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection. DO NOT proceed with transfection if cells are not doubling every 24 hours or are < 95% viable by trypan blue exclusion.
2. Incubate cells overnight at appropriate temperature and CO₂ levels (e.g. 37°C, 5-8% CO₂, shaking).

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

1. Seed cells at a density of 2 x 10⁶ cells/ml immediately before transfection. DO NOT proceed with transfections if cells are not doubling normally or at high viability.
2. Warm *TransIT-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 *TransIT-PRO*® Reagent. Mix gently by pipetting.
6. (Optional) Add ___ µl of PRO Boost Reagent. Mix gently by pipetting.
7. Incubate complexes at room temperature for 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 *TransIT-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 *TransIT-PRO*® Reagent with the CHOgro® Expression System, form transfection complexes in CHOgro® Complex Formation Solution.

Table 1. Volume scaling worksheet for DNA transfections with *TransIT-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
<i>TransIT-PRO</i> ® Reagent	1 µl	× _____ ml	= _____ µl
PRO Boost Reagent (optional)	0.5 µl	× _____ ml	= _____ µl

► Transfection Optimization

Determine the best *TransIT-PRO*® Reagent:DNA ratio for each cell type. Start with 1 µl of *TransIT-PRO*® Reagent per 1 µg of DNA. Vary the concentration of *TransIT-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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