*Trans*IT-PRO® Transfection Reagent

Quick Reference Protocol

Instructions for MIR 5720, 5730, 5740 and 5750

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/5740

SPECIFICATIONS

Storage	Store *Trans*IT-PRO® 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/5740***

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 CO_2 levels (e.g. 37°C, 5-8% CO_2, shaking).

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

1. Seed cells at a density of 2 × 10^6 cells/ml immediately before transfection. DO NOT proceed with transfections if cells are not doubling normally or are not at high viability.
2. Warm *Trans*IT-PRO® Reagent to room temperature and vortex gently.
3. Place ___ml of serum-free medium (e.g. Opti-MEM® or Opti-PRO™ SFM)* 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. Incubate complexes at room temperature for 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: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 *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® Reagent	1 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. *Trans*IT-PRO® Transfection Reagent is a key component of the CHOgro® Expression System (MIR 6260) and the CHOgro® High Yield Expression System (MIR 6270), which are optimized platforms for transient, high titer protein production in suspension CHO derived cells.

For additional optimization tips, see full protocol.

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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