SARTORIUS

Short Protocol

jetMESSENGER® Transfection Reagent

Short Protocol – mRNA Transfection

Day 0: Cell Seeding

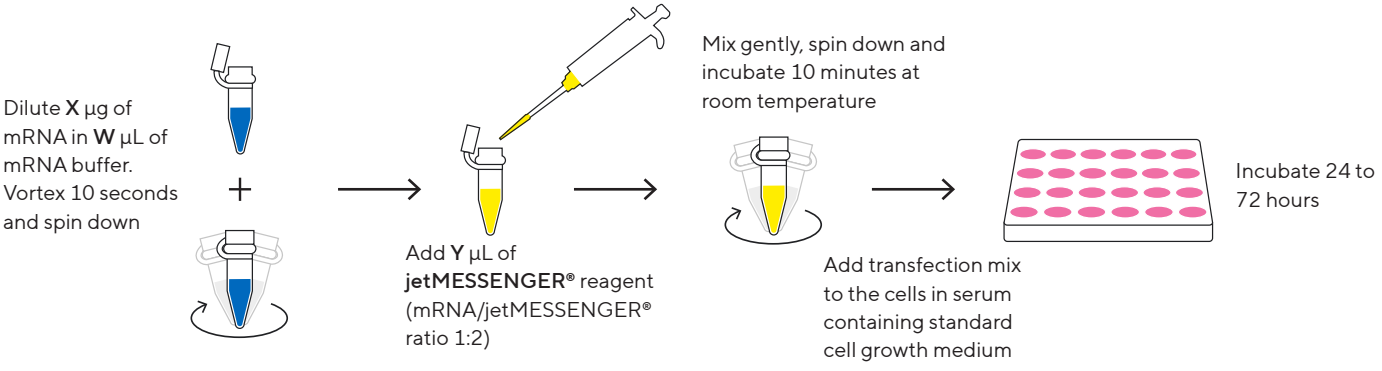
- Seed cells in **V** mL of standard cell growth medium according to the table below

Culture vessel	Number of cells*	V = volume of growth medium for cell seeding
96-well	12,500	0.125 mL
24-well	50,000	0.5 mL
12-well	100,000	1 mL
6-well/35 mm	200,000	2 mL
100 mm/flask 75 cm²	2×10^6	10 mL

Quantities per well, dish or flask.
*For specific cell type or suspension cells, please refer to the complete protocol.

Day 1: Transfection

- Perform transfection **in the standard cell growth medium**
- Use **jetMESSENGER® mRNA buffer only**
- Transfect cells at **60 – 80% confluency**



Culture vessel	W = volume of mRNA buffer	X = amount of mRNA added	Y = volume of jetMESSENGER® reagent
96-well	12.5 µL	0.1 µg	0.25 µL
24-well	50 µL	0.5 µg	1 µL
12-well	100 µL	1 µg	2 µL
6-well/35 mm	200 µL	2 µg	4 µL
100 mm/flask 75 cm²	1,000 µL	10 µg	20 µL

Quantities per well, dish or flask.

Day 2 – 3: Measure Gene Expression

See back page for optimization tips.

Download complete protocol on sartorius.com

Short Protocol – Optimization Tips

Protocol Optimization

- Test different mRNA amounts between 0.5X and 2X.
- Test different mRNA/jetMESSENGER® ratios, 1:2 to 1:3.
- For cell specific protocols, visit www.sartorius.com.

Culture vessel	W = volume of mRNA buffer	X = amount of mRNA added	Y = volume of jetMESSENGER® reagent
96-well	12.5 µL	0.1 ± 0.05 µg	0.25 ± 0.05 µL
24-well	50 µL	0.5 ± 0.1 µg	1 ± 0.2 µL
12-well	100 µL	1 ± 0.2 µg	2 ± 0.4 µL
6-well/35 mm	200 µL	2 ± 0.5 µg	4 ± 0.8 µL
100 mm/flask 75 cm^2	1,000 µL	10 ± 2.5 µg	20 ± 4 µL

Quantities per well, dish or flask.

Tips to Increase Cell Viability of Sensitive Cells

- Wash cells 4 hours after transfection.
- Ensure that the mRNA is diluted in the mRNA buffer provided by Polyplus-transfection®.
- Analyze transfection at an earlier time point (e.g., at 24 hours instead of 48 hours).
- Decrease the amount of mRNA added per well.
- Decrease the volume of jetMESSENGER® reagent.
- Use more stable chemically modified mRNA.
- Check if the expressed protein may cause toxicity. If this is the case, reduce the amount of mRNA.

Good mRNA Transfection Practices

- Store appropriately jetMESSENGER® (5 ± 3 °C) and the mRNA (- 80 °C).
- Ensure that the quality of your mRNA is optimal. Preferably use mRNA purchased from an oligo supplier, instead of homemade transcribed mRNA.
- Use a common reporter gene-encoding mRNA as a positive control (ex: Luciferase or GFP).
- Ensure the medium is permissive to the transfection.
- The use of chemically modified mRNA (Pseudouridine, 5' Methylcytosine, 5-methoxyuridine, etc...) could improve the transfection efficiency.
- Ensure that all reagents are RNAse-free.

⚠ NOTE:
Please refer to the complete protocol available on www.sartorius.com.

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