

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

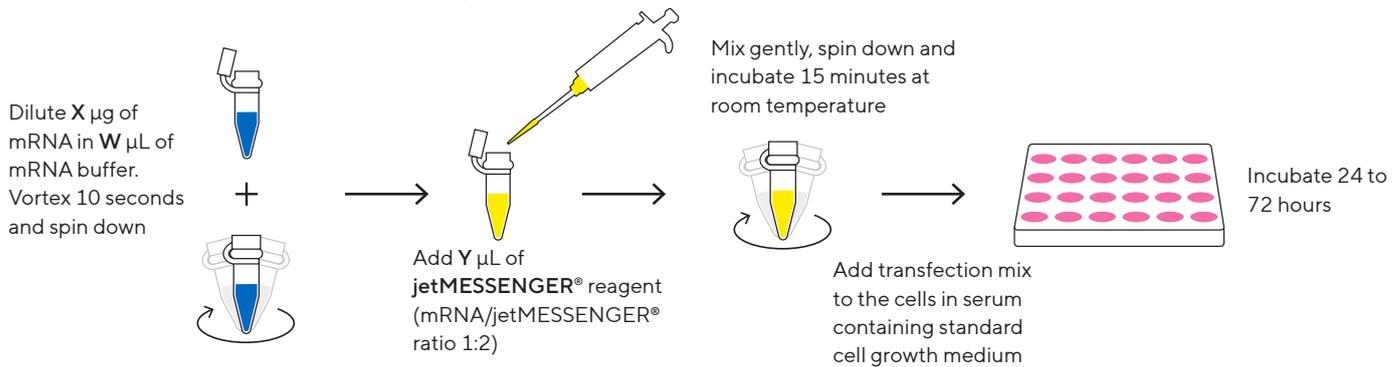
Culture vessel	Number of cells*	V = volume of growth medium for cell seeding [mL]
96-well	12,500	0.125
24-well	50,000	0.5
12-well	100,000	1
6-well/35 mm	200,000	2
100 mm/flask 75 cm ²	2 x 10 ⁶	10

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

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

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.

Germany

Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

 For further information, visit
sartorius.com