jetMESSENGER® transfection reagent

Short protocol – mRNA transfection

mRNA TRANSFECTION

Day 0: Cell seeding

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

Quantities per well, dish or flask

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 cm^2	2 x 10^6	10 mL

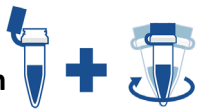
*For specific cell type or suspension cells, please refer to the complete protocol.

Day 1: Transfection

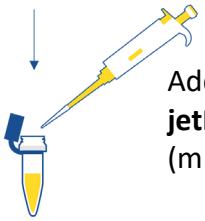
→ Perform transfection **in the standard cell growth medium**

→ Use jetMESSENGER® **mRNA buffer only**

→ Transfect cells at **60-80% confluency**



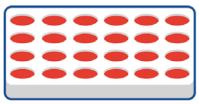
Dilute **X** µg of mRNA in **W** µL of mRNA buffer. Vortex 10 s and spin down



Add **Y** µL of **jetMESSENGER®** reagent (mRNA/jetMESSENGER® ratio 1:2)

Mix gently, spin down and incubate 10 min at RT

Add transfection mix to the cells in serum containing standard cell growth medium



Incubate 24 to 72 h

Quantities per well, dish or flask

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 cm^2	1000 µL	10 µg	20 µL

Day 2-3: Measure gene expression

See back page for optimization tips

Download complete protocol on https://myaccount.polyplus-transfection.com/

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jetMESSENGER® transfection reagent

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, check our online Cell Transfection Database:

http://www.polyplus-transfection.com/resources/cell-transfection-database/

Quantities per well, dish or flask

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	1000 µL	10 ± 2.5 µg	20 ± 4 µL

Tips to increase cell viability of sensitive cells

- Wash cells 4 h 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 h instead of 48 h).
- 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 when creating your account online at: https://myaccount.polyplus-transfection.com/.

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