jetMESSENGER® transfection reagent
Short protocol - mRNA Transfection

DAY 0: Cell seeding

→ Seed cells in V ml of serum containing medium according to the table below

Quantities per well, dish or flask

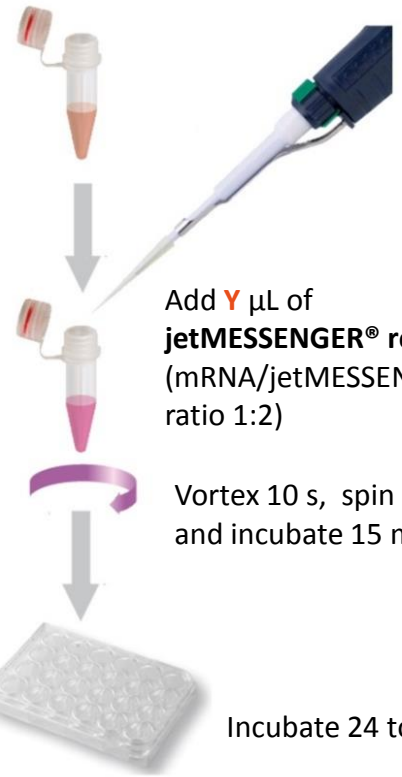
Culture vessel	Number of cells*	V = volume of growth medium for cell seeding
96-well	7 500 - 12 500	0.125 mL
24-well	12 000 - 50 000	0.5 mL
12-well	80 000 - 100 000	1 mL
6-well / 35 mm	150 000 - 200 000	2 mL
100 mm / flask 75 cm^2	2 x 10^6 - 4 x 10^6	10 mL

*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)

Vortex 10 s, spin down
and incubate 15 min at RT

Add transfection mix to the cells
in serum containing 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 http://www.polyplus-transfection.com/resources/product-literature/

Polyplus transfection® THE DELIVERY EXPERTS
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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.
- Check our online Cell Transfection Database for cell specific protocols at:
  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: For more information regarding experimental conditions, please refer to the complete protocol available online at: http://www.polyplus-transfection.com/resources/product-literature/

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