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

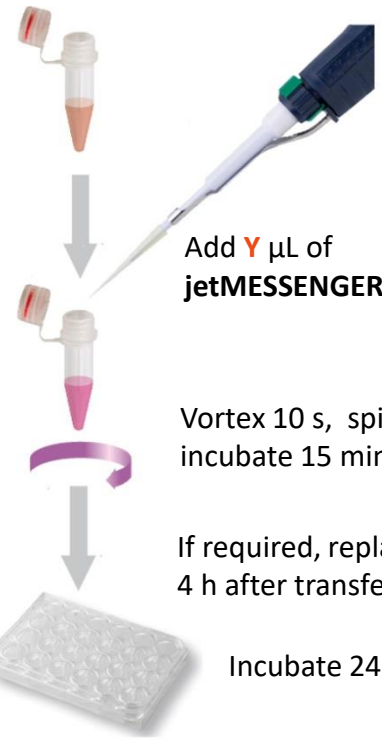
Culture vessel	Number of cells*	V seeding volume
24-well	7 000 – 50 000	0.5 ml
6-well / 35 mm	80 000 – 200 000	2 ml
100 mm / flask 75 cm^2	1 000 000 – 2 000 000	10 ml

*For suspension cells, please refer to the complete protocol.

DAY 1: Transfection = 1:2 mRNA to jetMESSENGER® reagent ratio

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

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



Add **Y** µL of
jetMESSENGER® reagent

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

Add transfection mix
to the cells in serum
containing medium

If required, replace medium 4 h after transfection

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
24-well	50 µL	0.5 µg	1 µL
6-well / 35 mm	200 µL	2 µg	4 µL
100 mm / flask 75 cm^2	1 000 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/

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jetMESSENGER® transfection reagent
Short protocol - Optimization Tips

Protocol Optimization

- Check our online Cell Transfection Database for cell specific protocols at: http://www.polyplus-transfection.com/resources/cell-transfection-database/
- Test different mRNA amounts between 0.5X and 2X
- Test different mRNA/jetMESSENGER® ratios, 1:2 to 1:3

Quantities per well, dish or flask

Culture vessel	W volume of mRNA buffer	X amount of mRNA added	Y volume of jetMESSENGER® reagent
24-well	50 µL	0.4 – 0.6 µg	0.8 – 1.2 µL
6-well / 35 mm	200 µL	1.5 – 2.5 µg	3.2 – 4.8 µL
100 mm / flask 75 cm^2	1 000 µL	7.5 – 12.5 µg	16 – 24 µL

Tips to increase cell viability of sensitive cells

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

Good mRNA Transfection Practices

- Store appropriately jetMESSENGER® (4°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, 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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