jetMESSENGER®

in vitro mRNA transfection reagent

PROTOCOL

Description

jetMESSENGER® is a novel powerful transfection reagent manufactured at Polyplus-transfection®. jetMESSENGER® has been specifically designed for high mRNA transfection efficiency in usually difficult to transfect cells such as primary cells, cancer cell lines, neurons and stem cells. jetMESSENGER® can also be used on a wide variety of easy to transfect cells. Transfection with jetMESSENGER® leads to very low cytotoxicity as it requires low amounts of mRNA and low volumes of reagent.

1 Transient mRNA transfection protocol

1.1 Cell seeding

For optimal mRNA transfection conditions, we recommend using cells which are 60 to 80% confluent at the time of transfection. Typically, for experiments in 24-well plates, 50 000 adherent cells or 100 000 suspension cells are seeded per well in 0.5 mL of cell growth medium 24 h prior to transfection. For other culture formats, refer to Table 1. Some cells require to be seeded several days prior transfection, especially primary cells; for more details about seeding various cell lines, refer to Table 2.

jetMESSENGER® is compatible with the presence of serum and antibiotics therefore you may use serum and antibiotic containing medium during the entire experiment.

Table 1. Recommended seeding conditions

Culture vessel	Adherent cell number	Suspension cell number	Surface area per well (cm^2)	Volume of medium per well to seed the cells (mL)
96-well	12 500	25 000	0.3	0.125
24-well	50 000	100 000	1.9	0.5
12-well	100 000	200 000	3.8	1
6-well/35 mm	200 000	400 000	9.4	2
60 mm / flask 25 cm^2	800 000	1.6×10^6	20 - 25	5
100 mm / flask 75 cm^2	2×10^6	4×10^6	60 - 75	10
150 mm / flask 175 cm^2	5×10^6	1×10^7	150 - 175	20

Table 2. Recommended seeding conditions for various cells

Cell type	Cells	Number of cells to seed per well of a 24-well plate	Number of plating days before transfection (day)
Epithelial	Caco-2	40 000	1
	MCF 10A	80 000	1
	MCF7	50 000	1
	MDCK	40 000	1
	U-87 MG	50 000	1
Fibroblast	BJ	20 000	1
	MEF	12 000	**3**
	IMR-90	50 000	1
Hepatocyte	Hep G2	100 000	1
Human stem cells	hMSC	12 000	**3**
Lymphocyte	Jurkat	100 000	1
	K-562	100 000	1
Monocyte	THP-1	100 000	1
Mouse stem cells	mES	50 000	**3**
Primary cells	Monocytes	400 000	1
	Dendritic cells *	400 000	**7-11**
	Macrophages *	400 000	**7-11**

*Obtained from differentiation and maturation of monocytes

1.2 mRNA Transfection Protocol

The following conditions are given per well of a 24-well plate. For other culture formats, please refer to Table 3.

1. Seed cells according to Tables 1 and 2. Specific conditions for many cells are available in Polyplus-transfection® Database: http://www.polyplus-transfection.com/resources/cell-transfection-database/
2. On the day of transfection, dilute 0.5 µg mRNA into 50 µL jetMESSENGER® mRNA buffer (supplied). Mix by vortexing, spin down briefly.
3. Vortex jetMESSENGER® reagent for 5 sec and spin down before use.
4. Add 1 µL jetMESSENGER®, mix by vortexing, spin down briefly.
5. Incubate for 15 min at RT.
6. Add 50 µL of transfection mix per well dropwise onto the cells in growth medium (containing serum or not) and/or additives, and distribute evenly.
7. Gently rock the plate back and forth and from side to side.
8. Perform analysis 24 - 48 h later.

Table 3. mRNA transfection guidelines per well according to the cell culture vessel

Culture vessel	Volume of mRNA buffer (µL)	Amount of mRNA (µg)	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
60 mm / flask 25 cm^2	500	4 ± 1	8 ± 1.6
100 mm / flask 75 cm^2	1000	10 ± 2.5	20 ± 4

NOTES:

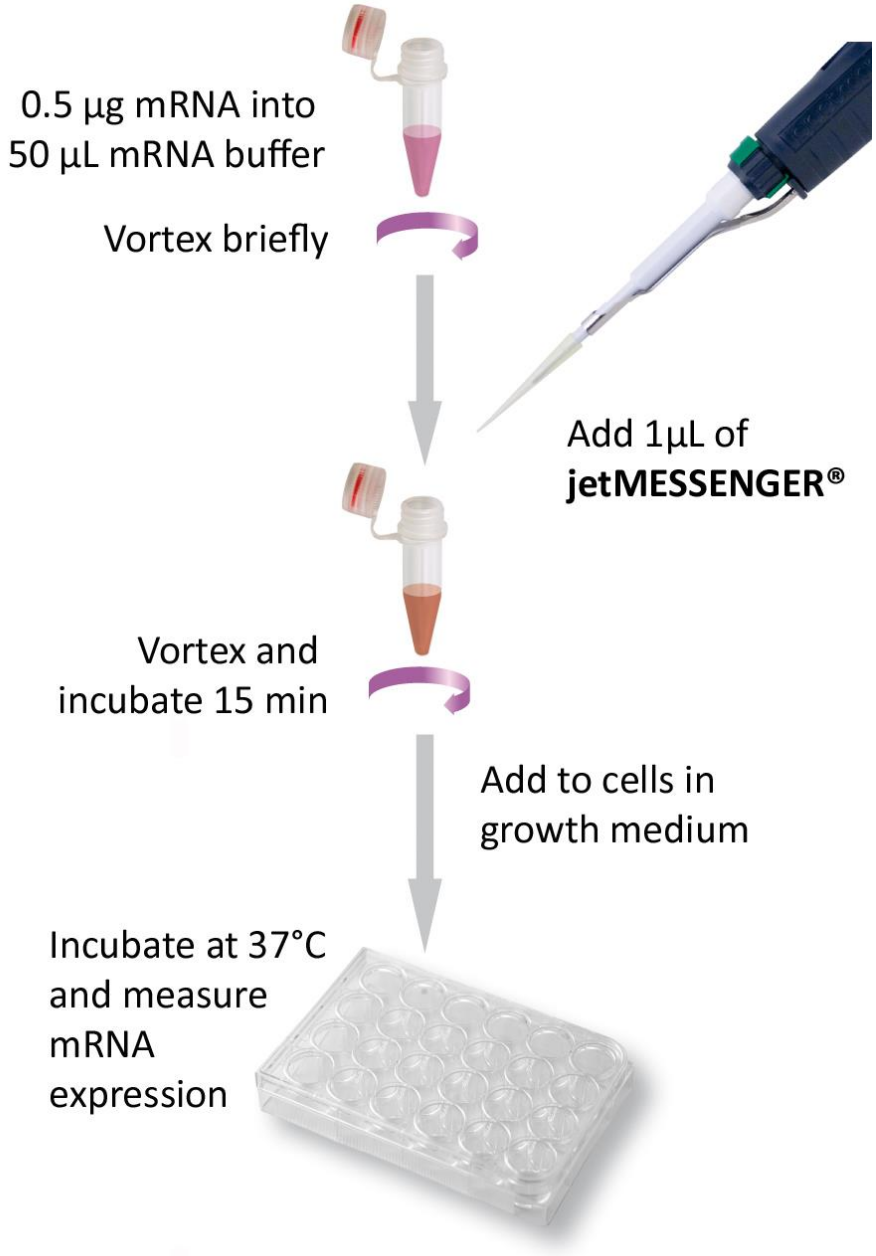
The provided mRNA buffer should be used for successful transfection with jetMESSENGER®.

Prepare a master mix of minimum 50 µL to allow accurate pipetting and homogenous preparation of the complexes.

Performing media change 4 h post-transfection may improve cell viability.

For optimal mRNA transfection conditions, we recommend using chemically modified mRNA. Transfection should be performed in a RNAse-free working-environment and mRNA should be diluted and aliquoted in RNAse-free water.

jetMESSENGER® Transfection in 24-well Plate



1.3 Optimization guidelines

Transfection conditions should be optimized for each cell line. You may refer to the optimized conditions for various cell lines detailed in **Table 2**, and on our online cell transfection database (http://www.polyplus-transfection.com/resources/cell-transfection-database/).

You may adjust the volume of reagent and/or the amount of mRNA. The volume of jetMESSENGER® may range between **1.6 - 2.4 µL per µg of mRNA** depending on the transfected cell line. The amount of mRNA may range between 0.5 X and 2 X, X being the amount indicated in Table 3.

Browse our cell transfection database to find the optimized conditions according to your cell line:

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

2 Transient mRNA reverse transfection protocol

In this procedure, the transfection mix is prepared as a mastermix, which is distributed into wells and cells are subsequently added.

2.1 Cell Preparation

The day of transfection, trypsinize cells and resuspend them in growth medium containing serum and antibiotics, at the recommended cell density according to Table 4.

Typically, for experiments in 24-well plates, a cell solution of 2 x 10^5 adherent cells/ml or 4 x 10^5 suspension cells/ml is prepared in culture medium on the day of transfection. 0.5 ml of cell suspension is added per well to the complexes. For other culture formats, refer to Table 4. For more details about seeding various cell lines, refer to Table 5.

jetMESSENGER® is compatible with the presence of serum and antibiotics, therefore you may use serum and antibiotic containing medium during the entire experiment.

Table 4. Recommended seeding cell density

Culture vessel	Adherent cell number per well	Adherent cell density (cells/mL)	Suspension cell number per well	Suspension cell density (cells/mL)	Volume of cell suspension per well (mL)
96-well	25 000	2 x 10^5	50 000	4 x 10^5	0.125
24-well	100 000		200 000		0.5
12-well	200 000		400 000		1
6-well/35 mm	400 000		800 000		2

Table 5. Recommended seeding density for various cells

Cell type	Cells	Number of cells to seed per well of a 24-well plate	Cell density (cells/mL)
Epithelial	Caco-2	80 000	1.6 x 10^5
	HeLa	100 000	2 x 10^5
	MCF 10A	160 000	3.2 x 10^5
	MCF7	100 000	2 x 10^5
	MDCK	80 000	1.6 x 10^5
	U-87 MG	100 000	2 x 10^5
Fibroblast	BJ	40 000	0.8 x 10^5
	IMR-90	100 000	2 x 10^5
Hepatocyte	Hep G2	200 000	4 x 10^5
Lymphocyte	Jurkat	200 000	4 x 10^5
	K-562	200 000	4 x 10^5
Monocyte	THP-1	200 000	4 x 10^5

2.2 mRNA Reverse Transfection Protocol

The following conditions are given per well of a 24-well plate. For other culture formats, please refer to Table 6.

1. On the day of transfection, dilute 0.5 µg mRNA into 50 µL jetMESSENGER® mRNA buffer (supplied). Mix by vortexing, spin down briefly.
2. Vortex jetMESSENGER® reagent for 5 sec and spin down before use.
3. Add 1 µL jetMESSENGER®, mix by vortexing, spin down briefly.
4. Incubate for 15 min at RT.
5. Add 50 µL of transfection mix into each well.
6. Add 0.5 mL of cell suspension to each well, according to Tables 4 and 5.
7. Gently rock the plate back and forth and from side to side.
8. Perform analysis 24 - 48 h later.

Table 6. mRNA reverse transfection guidelines per well according to the cell culture vessel

Culture vessel	Volume of mRNA buffer (µL)	Amount of mRNA (µg)	Volume of jetMESSENGER® Reagent (µL)	Volume of cell suspension per well (mL)
96-well	12.5	0.1 ± 0.05	0.25 ± 0.05	0.125
24-well	50	0.5 ± 0.1	1 ± 0.2	0.5
12-well	100	1 ± 0.2	2 ± 0.4	1
6-well/35 mm	200	2 ± 0.5	4 ± 0.8	2
60 mm / flask 25 cm^2	500	4 ± 1	8 ± 1.6	5
100 mm / flask 75 cm^2	1000	10 ± 2.5	20 ± 4	10

NOTE: the provided mRNA buffer should be used for successful transfection with jetMESSENGER®.

Prepare a master mix of minimum 50 µL to allow accurate pipetting and homogenous preparation of the complexes.

3 CRISPR/CAS9 applications

jetMESSENGER® is well suited for genome editing applications using Cas9 encoding mRNA co-transfected with guide RNA into mammalian cells.

For co-transfection of multiple nucleic acids, the total RNA amount added per well (or plate) should correspond to the RNA amounts indicated in **Table 3.**

The following conditions are given per well of a 24-well plate. For other culture format, please refer to **Table 3**.

1. Seed cells according to Tables 1 and 2. Specific conditions for many cells are available in Polyplus-transfection® Cell Database: http://www.polyplus-transfection.com/resources/cell-transfection-database/
2. On the day of transfection, dilute 0.5 µg mRNA into 50 µL mRNA Buffer (supplied). Mix by vortexing, spin down briefly
3. Vortex jetMESSENGER® reagent for 5 sec and spin down before use.
4. Add 1 µL jetMESSENGER®, mix by vortexing, spin down briefly.
5. Incubate for 15 min at RT.
6. Add 50 µL of transfection mix per well dropwise onto the cells in medium (containing serum or not), and distribute evenly.
7. Gently rock the plates back and forth and from side to side.
8. Perform analysis 48 - 72 h later.

If you would like to perform CRISPR/Cas9 transfection experiments using another type of nucleic acids such as plasmid DNA, please contact our technical support at support@polyplus-transfection.com.

4 Troubleshooting

Observations	Actions
Low mRNA transfection efficiency	Optimize the volume of jetMESSENGER® reagent and the amount of mRNA added per well. Increase the volume of jetMESSENGER® reagent first; if insufficient, increase the amount of mRNA according to **Table 3**. To adjust the volume of reagent and/or the amount of mRNA: - the volume of jetMESSENGER® may range between **1.6 - 2.4 µL per µg of mRNA** depending on the transfected cell line. - the amount of mRNA may range between 0.5X and 2X, X being the amount indicated in **Table 3**.
	Replace medium containing serum with serum-free medium (OptiMEM®) during transfection.
	Ensure the medium is permissive to the transfection.
	Ensure that the mRNA is diluted in the provided mRNA buffer by Polyplus-transfection®.
	Use a common reporter gene-encoding mRNA as a positive control (ex: Luciferase or GFP).
	Ensure that the quality of your mRNA is optimal. Preferably use mRNA purchased from an oligo supplier, instead of homemade transcribed mRNA.
	The use of chemically modified mRNA (Pseudouridine, 5' Methylcytosine, etc…) could improve gene expression.
	Ensure that all reagents are RNAse-free.
Cellular toxicity	Analyze transfection at an earlier time point (e.g. at 24 h instead of 48 h).
	Replace medium 4 h after transfection.
	Decrease the amount of mRNA added per well.
	Ensure that the mRNA is diluted in the provided mRNA buffer.
	Decrease the volume of jetMESSENGER® reagent.
	Ensure that the mRNA used is chemically modified.
	Check if the expressed protein may cause toxicity. If the expressed protein is toxic for the cells, reduce the amount of mRNA.

5 Product Information

5.1 Ordering information

Ref. N°	jetMESSENGER® Reagent	mRNA Buffer
150-01	0.1 mL	10 mL
150-07	0.75 mL	60 mL
150-15	1.5 mL	2 x 60 mL

5.2 Additional Buffer

jetMESSENGER® reagent is provided with an optimized sterile buffer (mRNA buffer). This buffer **must** be used to ensure successful transfection experiments.

5.3 Content

1.5 mL of jetMESSENGER® transfection reagent is sufficient to perform up to 1500 transfections in a 24-well plate and 375 transfections in 6-well plate format.

5.4 Reagent use and Limitations

For research use only. Not for use in humans.

5.5 Quality control

Every batch of jetMESSENGER® mRNA transfection reagent is tested in-house by mRNA transfection of CaCo-2 cells with a GFP-expressing mRNA. Each vial of reagent is provided with Certificate of Analysis.

5.6 Formulation and Storage

jetMESSENGER® and its buffer are shipped at room temperature but should be stored at 4°C upon arrival to ensure long term stability. jetMESSENGER®, as guaranteed and indicated in the Certificate of Analysis, is stable at least for 6 months (150-01) to at least one year (other packaging sizes) when stored appropriately.

Polyplus-transfection® has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution and customer support.

5.7 Trademarks

Polyplus-transfection and jetMESSENGER are registered trademarks of Polyplus-transfection.

How to cite us: “jetMESSENGER® (Polyplus-transfection S.A, Illkirch, France)”

5.8 Technical Assistance and Scientific Advice

Contact the friendly Polyplus technical support *via*:

- The Polyplus website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87