SARTORIUS

Protocol

jetMESSENGER®

In Vitro mRNA Transfection Reagent

Description

jetMESSENGER® is a novel powerful transfection reagent 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 hours 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	7,500 – 25,000	25,000	0.3	0.125
24-well	40,000 – 100,000	100,000	1.9	0.5
12-well	80,000 – 150,000	200,000	3.8	1
6-well/35 mm	150,000 – 400,000	400,000	9.4	2
60 mm/flask 25 cm^2	200,000 – 850,000	1.6×10^6	20 – 25	5
100 mm/flask 75 cm^2	1×10^6 – 4×10^6	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 formats, please refer to Table 3.

1. Seed cells according to Tables 1 and 2. Specific conditions for many cells are available on www.sartorius.com.
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 seconds and spin down before use.
4. Add 1 µL jetMESSENGER®, mix gently by up-and-down pipetting.
5. Incubate for 15 minutes at room temperature.
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 to 48 hours later.

Table 3: *mRNA Transfection Guidelines per Well According to the Cell Culture Vessel*

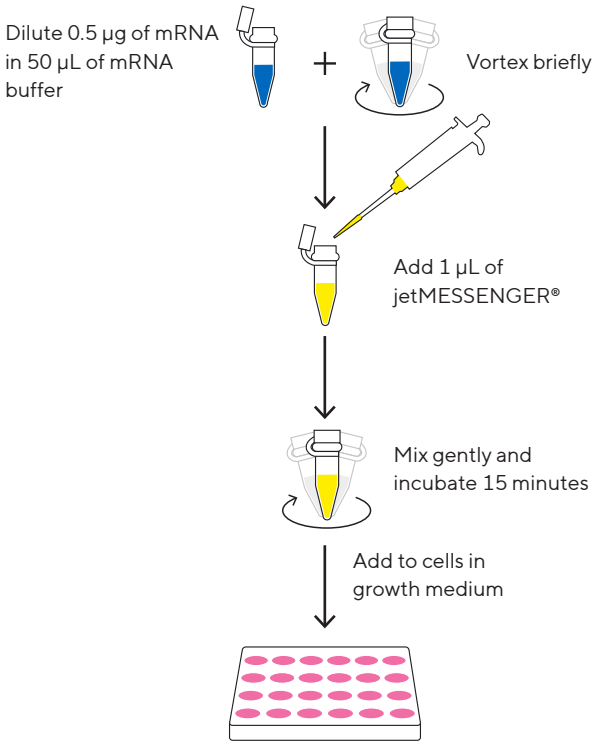
Culture vessel	Volume of mRNA buffer [µL]	Amount of mRNA [µg]	Volume of jetMESSENGER® reagent [µL]
96-well	12.5	0.1 ± 0.05	0.25 ± 0.05
24-well	50	0.5 ± 0.1	1 ± 0.2
12-well	100	1 ± 0.2	2 ± 0.4
6-well/35 mm	200	2 ± 0.5	4 ± 0.8
60 mm/flask 25 cm^2	500	4 ± 1	8 ± 1.6
100 mm/flask 75 cm^2	1000	10 ± 2.5	20 ± 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.

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



Incubate at 37 °C and measure mRNA expression

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 at www.sartorius.com.

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 0.5X and 2X, X being the amount in µg indicated in Table 3.

2 Transient mRNA Reverse Transfection Protocol

In this procedure, the transfection mix is prepared as a master mix, 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. jetMESSENGER® is compatible with the presence of serum and antibiotics, therefore you may use serum and antibiotic containing medium during the entire experiment.

Typically, for experiments in 24-well plates, a cell solution of 2×10^5 adherent cells/mL or 4×10^5 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.

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	2×10^5	50,000	4×10^5	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	1.6×10^5
	HeLa	100,000	2×10^5
	MCF 10A	160,000	3.2×10^5
	MCF7	100,000	2×10^5
	MDCK	80,000	1.6×10^5
	U-87 MG	100,000	2×10^5
Fibroblast	BJ	40,000	0.8×10^5
	IMR-90	100,000	2×10^5
Hepatocyte	Hep G2	200,000	4×10^5
Lymphocyte	Jurkat	200,000	4×10^5
	K-562	200,000	4×10^5
Monocyte	THP-1	200,000	4×10^5

2.2 mRNA Reverse Transfection Protocol

The following conditions are given per well of a 24-well plate. For other 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 seconds and spin down before use.
3. Add 1 µL jetMESSENGER®, mix gently by up-and-down pipetting.
4. Incubate for 15 minutes at room temperature.
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 to 48 hours 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® [µL]	Volume of cell suspension per wells [mL]
96-well	12.5	0.1 ± 0.05 µL	0.25 ± 0.05 µL	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 cm²	500	4 ± 1	8 ± 1.6	5
100 mm | flask 75 cm²	1,000	10 ± 2.5	20 ± 4	10

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

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 formats, please refer to Table 3.

1. Seed cells according to Tables 1 and 2. Specific conditions for different cell types are available at www.sartorius.com.
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 seconds and spin down before use.
4. Add 1 µL jetMESSENGER®, mix gently by up-and-down pipetting.
5. Incubate for 15 minutes at room temperature.
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 to 72 hours later.

If you would like to perform CRISPR/Cas9 transfection experiments using another type of nucleic acid such as plasmid DNA, please contact us at askascientist@sartorius.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 (Opti-MEM™) during transfection. ▪ Ensure the medium is permissive to the transfection. ▪ Ensure that the mRNA is diluted in the provided mRNA buffer. ▪ 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 hours instead of 48 hours). ▪ Replace medium 4 hours 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

Part number	jetMESSENGER® reagent vial size	buffer
101000056	0.1 mL	10 mL
101000005	0.75 mL	60 mL

5.2 Provided 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 1,500 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. Certificates of Analysis are available online in the MySartorius portal on www.sartorius.com.

5.6 Formulation and Storage

jetMESSENGER® and its buffer should be stored at 5 ± 3 °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 (101000056) to at least one year (other packaging sizes) when stored appropriately.

5.7 Trademarks

Polyplus-transfection® and jetMESSENGER® are registered trademarks of Polyplus-transfection S.A. Opti-MEM™ is a trademark of Life Technologies, Inc.

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