Transfecting cryopreserved human and fresh rat hepatocytes with GFP mRNA using Lipofectamine MessengerMAX reagent

Introduction

Transfection, a method to introduce exogenous DNA or RNA into eukaryotic cells, is critical for the study of gene expression. Stem and primary cells, in addition to established cell lines, can be successfully transfected using biological, chemical, or physical methods. Choosing the appropriate transfection method for a particular cell type and application is vital to the success of an experiment. Thermo Fisher Scientific offers numerous transfection products, including lipid-based reagents for difficult-to-transfect cells such as primary hepatocytes.

Primary hepatocytes are the premier *in vitro* tool used in liver disease modeling and functional studies, fostering the advancement of novel drug therapies to treat human diseases such as type 2 diabetes and non-alcoholic steatohepatitis (NASH). To enable more biologically relevant research, transfection of messenger RNA (mRNA) can now be done effectively with low toxicity to cells using lipid-based reagents. To maximize results and help ensure repeatability, we recommend Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent. This reagent is optimized for mRNA delivery into primary cells, delivers an outstanding >60% transfection efficiency (over 2-fold improvement compared to other lipid-based reagents), and guarantees faster protein expression than DNA transfection.

In this application note, we demonstrate mRNA transfection efficiency through GFP expression using Lipofectamine MessengerMAX reagent in cryopreserved human hepatocytes and fresh rat hepatocytes.

Materials and methods

Transfection of cryopreserved human hepatocytes

Gibco™ cryopreserved human hepatocytes (Cat. No. HMCPTS) were transfected with mRNA using **Lipofectamine MessengerMAX reagent (Cat. No. LMRNA008)** in 24- and 96-well formats on **Gibco™ collagen I–coated plates (Cat. No. A1142802** and **A1142803)**. Cells were thawed and plated following the user guide for cryopreserved hepatocytes **(Pub. No. MAN0018379)**. The thawing medium was **Gibco™ Hepatocyte Thaw Medium (Cat. No. CM7500)**, and the plating medium consisted of **Gibco™ Williams' E Medium (Cat. No. A1217601)** and **Gibco™ Primary Hepatocyte Thawing and Plating Supplements (Cat. No. CM3000)**. The incubation medium consisted of **Williams' E Medium (Cat. No. A1217601)** and **Gibco™ Primary Hepatocyte Maintenance Supplements (Cat. No. CM4000)**.

For mRNA transfection in the 24-well format, 1.1 x 10^5 cryopreserved hepatocytes were seeded in each well on a collagen I–coated plate. Leaving the newly seeded hepatocytes undisturbed for at least 4 hours, the plating medium was replaced with incubation medium 6–8 hr after thawing and plating. The incubation medium was changed 24 hr after plating and followed by immediate dosing of each well with a 50 µL complex containing 500 ng of mRNA and 1.5 µL of Lipofectamine MessengerMAX reagent, added dropwise to the plate followed by a gentle swirl. The transfection procedure for the 96-well format was similar, except that each well was seeded with 2.2 x 10^4 cryopreserved hepatocytes and dosed with a 10 µL complex containing 100 ng of mRNA and 0.3 µL of Lipofectamine MessengerMAX reagent. All fluorescence and phase-contrast images were acquired 24 hr posttransfection.

Transfection of fresh rat hepatocytes

Fresh rat hepatocytes were transfected with mRNA using Lipofectamine MessengerMAX reagent in a 24-well format on a collagen I–coated plate. A total of 3 x 10^5 hepatocytes were seeded per well in Williams' E Medium and Primary Hepatocyte Maintenance Supplements. The incubation medium was changed 24 hr after plating and followed by immediate dosing of each well with a 50 µL complex containing 500 ng of mRNA and 1.5 µL of Lipofectamine MessengerMAX reagent. All fluorescence and phase-contrast images were acquired 24 hr posttransfection. The transfection procedure used for each format was based on the user guide (Pub. No. MAN0010803, Figure 1).

Timeline	Step	Procedure details (two-reaction optimization)		
		Component	96-well	24-well
Day 0	1  Seed cells to be 70–90% confluent at transfection	Adherent cells	1–4 x 10^4	0.5–2 x 10^5
	2 Diluted Lipofectamine MessengerMAX reagent  Dilute Lipofectamine MessengerMAX reagent in Opti-MEM medium (2 tubes)—mix well	Opti-MEM medium	5 µL x 2	25 µL x 2
		Lipofectamine MessengerMAX reagent	0.15 and 0.30 µL	0.75 and 1.50 µL
	3 Vortex 2–3 sec  Incubate	Incubate diluted Lipofectamine MessengerMAX reagent in Opti-MEM medium for 10 min at room temperature		
Day 1	4 Diluted mRNA  Prepare diluted mRNA master mix by adding mRNA to Opti-MEM medium—mix well	Opti-MEM medium	10 µL	50 µL
		mRNA (0.5–5 µg/µL)	0.2 µg	1 µg
	5  Add diluted mRNA to each tube of diluted Lipofectamine MessengerMAX reagent (1:1 ratio)	Diluted mRNA	5 µL	25 µL
		Diluted Lipofectamine MessengerMAX reagent	5 µL	25 µL
	6  Incubate	Incubate for 5 min at room temperature		
	7  Add mRNA–lipid complex to cells	Component (per well)	96-well	24-well
		mRNA–lipid complex	10 µL	50 µL
		mRNA	100 ng	500 ng
		Lipofectamine MessengerMAX reagent	0.15 and 0.30 µL	0.75 and 1.50 µL
Day 2–3	8  Visualize and analyze transfected cells	Incubate cells for 1–2 days at 37°C, then analyze transfected cells.		

Figure 1. Protocol for transfection of mRNA in 24-well or 96-well format. Replace the plating medium with incubation medium 6–8 hr after seeding of cryopreserved human hepatocytes. Change the incubation medium 24 hr after seeding of cryopreserved human hepatocytes or fresh rat hepatocytes (just prior to transfection). Since transfection occurs 50–90 min after dosing, the medium can be changed as early as 4 hr posttransfection. Gibco™ Geltrex™ matrix can be overlaid posttransfection.

Results and conclusions

We have verified a method to transfect cryopreserved human hepatocytes and fresh rat hepatocytes with GFP mRNA. Data generated in this study demonstrate the scalability of transfecting cryopreserved hepatocytes in 24- and 96-well formats. Using the recommended protocol, cryopreserved human hepatocytes dosed with GFP mRNA and Lipofectamine MessengerMAX reagent in 24- and 96-well formats express GFP and exhibit ideal confluency with no morphological change after mRNA transfection (Figure 2). These data also demonstrate that fresh rat hepatocytes dosed with GFP mRNA and Lipofectamine MessengerMAX reagent in a 24-well format express GFP and exhibit ideal confluency with no morphological change after mRNA transfection.

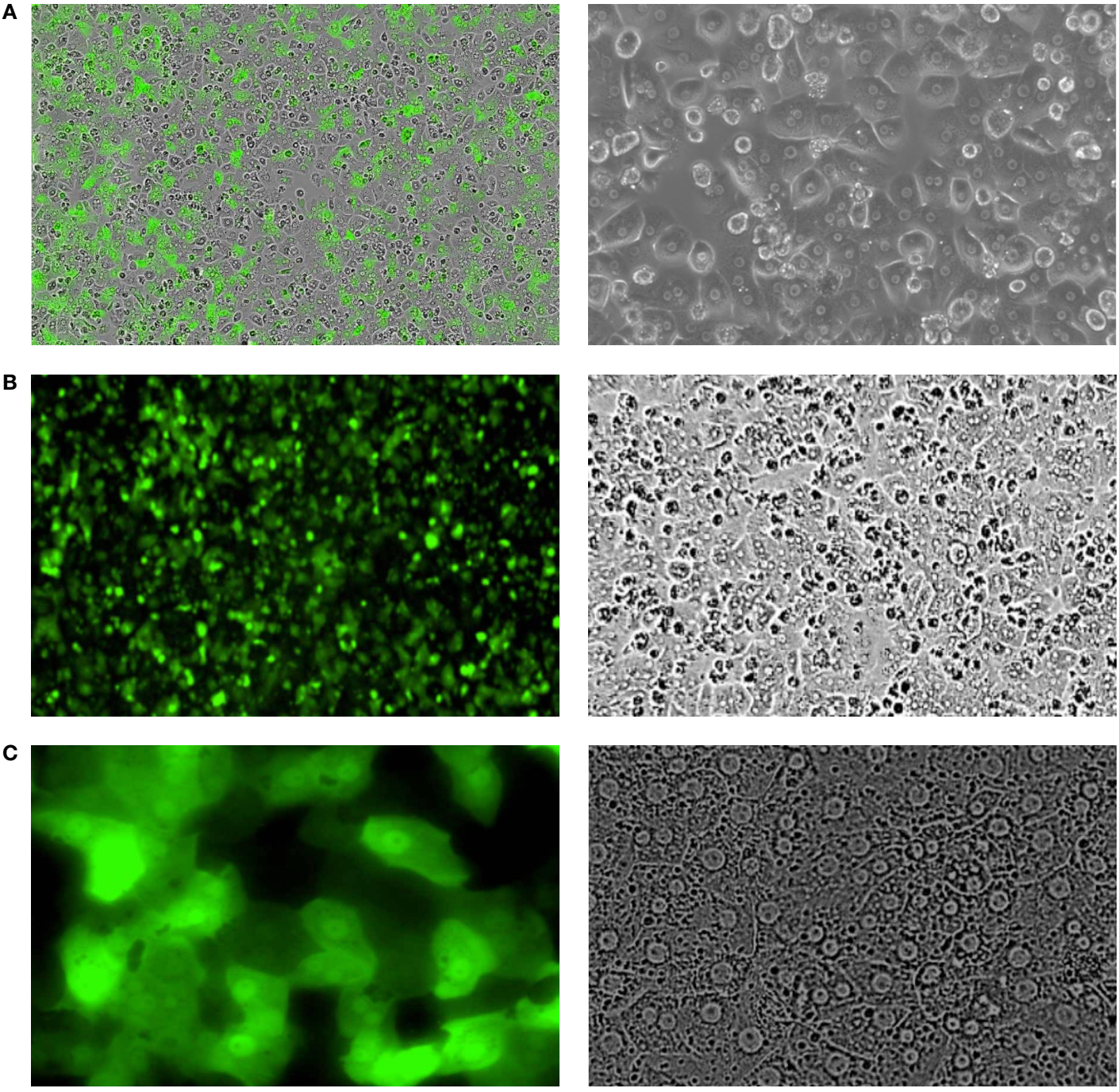

Figure 2. Transfection of cryopreserved human hepatocytes and fresh rat hepatocytes. All experiments show GFP expression and no morphological change after mRNA transfection using Lipofectamine MessengerMAX reagent. Fluorescence (left) and phase-contrast (right) images were acquired 24 hr posttransfection. **(A)** Cryopreserved human hepatocytes, 24-well format (500 ng GFP mRNA, 1.5 µL Lipofectamine MessengerMAX reagent). **(B)** Cryopreserved human hepatocytes, 96-well format (100 ng GFP mRNA, 0.3 µL Lipofectamine MessengerMAX reagent). **(C)** Fresh rat hepatocytes, 24-well format (500 ng GFP mRNA, 1.5 µL Lipofectamine MessengerMAX reagent).

Ordering information

Product	Quantity	Cat. No.
Lipofectamine MessengerMAX Transfection Reagent	0.75 mL	**LMRNA008**
Hepatocyte Thaw Medium	45 mL	**CM7500**
Primary Hepatocyte Maintenance Supplements	1 kit	**CM4000**
Williams' E Medium, no phenol red	500 mL	**A1217601**
Collagen I, Coated Plate, 24 well	5 plates	**A1142802**
Collagen I, Coated Plate, 96 well	5 plates	**A1142803**
Human Plateable Hepatocytes, Induction Qualified	1.5 mL	**HMCPIS**
Human Plateable Hepatocytes, Transporter Qualified	1.5 mL	**HMCPTS**
Human Plateable Hepatocytes, Metabolism Qualified	1.5 mL	**HMCPMS**
HEP10, Pooled Human Cryopreserved Hepatocytes	1.5 mL	**HMCS10**

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