HepG2

Liver cancer cells

Complete growth medium

Component	Cat. No.
Gibco™ DMEM with GlutaMAX™ Supplement	A3635201
10% Gibco™ FBS	A3160401

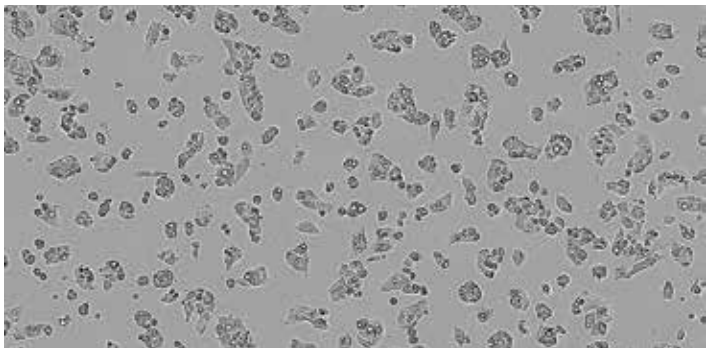
Proper culture techniques and procedures are an essential part of ensuring successful transfection. Subculturing, also referred to as passaging, is the removal of medium and transfer of cells from a culture into fresh growth medium, in order to propagate the cells.

Tip: HepG2 cells tend to grow in clusters. It is critical to ensure dissociation has occurred to yield a single-cell suspension when passaging and plating the cells. Having a single-cell suspension is also very important to ensure efficient transfection.

Passaging

- Maintain cells in T-75 flasks.
- Use Gibco™ TrypLE™ dissociation reagent.
- Passage cells every 3–4 days to ensure that they do not enter senescence.
- Transfection of cells should be performed only between passages 5 and 25 post-thaw.
- If designing an experiment that involves transfection, ensure that setup coincides with a cell passage.
- Plate cells for transfection only 1 day before the experiment.

Tip: Trypsinize HepG2 cells with TrypLE dissociation reagent for 10–15 min in a 37°C incubator. Then add 10 mL of growth medium and use a 10 mL serological pipette with a 200 µL tip on the end to pipette the cell suspension up and down at least 5 times. This step is the most critical to obtain single cells for accurate counting and plating.



Seeding cells for transfection

- The day before transfection, dissociate cells that are 80–90% confluent in a T-75 flask.
- Count the cells using standard trypan blue exclusion.
  - **Important:** The cell number and concentration determined can vary significantly depending on what method is used for counting; it is important to be consistent and use a single method throughout an experiment.
- The cell culture must have >90% viability and be 75–80% confluent on the day of transfection.
  - **Important:** If cells are not at the right confluence, do not wait until the next day to perform transfection, as this can significantly affect transfection efficiency.
- Seed 1.1 x 10^5 cells in 500 µL growth medium for a single well of a 24-well plate.

Transfection protocol

Transfection components

Component	Cat. No.
Invitrogen™ Lipofectamine™ 3000 Transfection Reagent	L3000008
Gibco™ Opti-MEM™ I Reduced Serum Medium	A3635101
Thermo Scientific™ Nunc™ 24-Well Cell Culture–Treated Multidish	142475

On the day of transfection, which should be 1 day following cell plating, perform the following steps, which have been optimized for a single well of a 24-well plate using Lipofectamine 3000 Transfection Reagent:

Step	Tube	Complexation components	Amount per well (24-well)
1	Tube 1	Opti-MEM I medium	25 µL
		Lipofectamine 3000 reagent	0.75 µL
2	Tube 2	Opti-MEM I medium	25 µL
		DNA amount (DNA concentration should be 0.5–5 µg/µL)	500 ng
		P3000™ reagent	1 µL
3	Add tube 2 solution to tube 1 and mix well		
4	Incubate mixture from step 3 at room temperature for 10–15 min		
5	Add 50 µL of complex from step 4 to cells; gently swirl plate to ensure homogeneous distribution of complex to the entire well		

Transfection efficiency analysis

At 48 hr following transfection of a GFP reporter construct, cells were evaluated via microscopy and flow cytometry. To assess transfection efficiency, cells were first visualized via fluorescence microscopy for qualitative assessment of protein expression, morphology, and viability (Figure 1). Cells were then prepared for flow cytometry by aspirating the medium and replacing it with 250 µL of a 7:3 mixture of TrypLE reagent:1X DPBS. Cells were incubated at 37°C for 10 min and then pipetted up and down to ensure single cells for flow cytometry analysis.

Tips and tricks

- Decreasing the serum content of the culture medium (to <10%) at the time of transfection is acceptable, but replace with complete growth medium within 4–24 hr posttransfection.
- Antibiotics can be used during transfection.
- Prior to flow cytometry, visualize cells under a bright-field microscope to verify dissociation following incubation with TrypLE reagent.

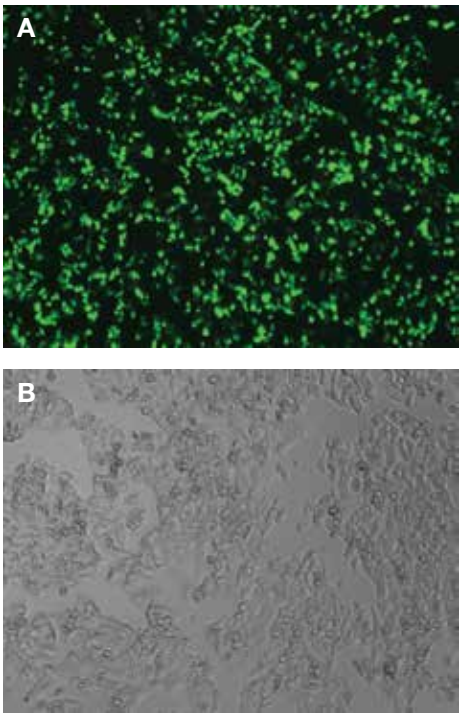


Figure 1. Posttransfection analysis of cells. **(A)** Fluorescence and **(B)** bright-field images demonstrating 65–75% transfection efficiency.

invitrogen

Scaling up or down Lipofectamine 3000 reagent transfections

Use the following table to scale the volumes for your transfection experiment. The most common sizes are listed below.

Culture vessel	Multiplication factor*	Shared reagents		DNA transfection			siRNA transfection	
		Growth medium	Opti-MEM medium for complexing	DNA	P3000 reagent	Lipofectamine 3000 reagent**	siRNA	Lipofectamine 3000 reagent**
96-well	0.2	100 µL	2 x 5 µL	100 ng	0.1 µL	0.15 µL	3 pmol	0.3 µL
48-well	0.5	250 µL	2 x 12.5 µL	0.25 µg	0.5 µL	0.375 µL	7.5 pmol	0.75 µL
24-well	1	500 µL	2 x 25 µL	0.5 µg	1 µL	0.75 µL	15 pmol	1.5 µL
12-well	2	1 mL	2 x 50 µL	1 µg	2 µL	1.5 µL	30 pmol	3 µL
6-well	5	2 mL	2 x 125 µL	2.5 µg	5 µL	3.75 µL	75 pmol	7.5 µL
60 mm	11.05	5 mL	2 x 250 µL	5.5–11 µg	11–22 µL	8.5 µL	166 pmol	17 µL
10 cm	28.95	10 mL	2 x 500 µL	14–28 µg	28–56 µL	22 µL	434 pmol	43 µL
T-75	39.47	15 mL	2 x 750 µL	20–40 µg	40–80 µL	30 µL	592 pmol	59 µL
T-175	92.11	35 mL	2 x 1.75 mL	46–96 µg	92–180 µL	69 µL	1,382 pmol	138 µL

* After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.
** Optimum amount needed is determined from the protocol for Lipofectamine 3000 Transfection Reagent.

For your convenience, the essential components of this protocol are now available in the Gibco™ Liver Cancer Starter Kit. The kit includes: basal medium, FBS, Lipofectamine 3000 reagent, Opti-MEM medium, and TrypLE reagent. The kit is available at **thermofisher.com/cancercellculture**

Find out more at **thermofisher.com/3000**

ThermoFisher SCIENTIFIC