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HepG2 Transfection Protocol (Kit for Hepatocellular Carcinoma Cells, HB-8065)

Catalog No. 2067 Size: 0.5 ml
Catalog No. 2068 Size: 1.5 ml
Catalog No. 2069 Size: 8.0 ml

Contents and Shipping:
HepG2 Transfection Kit includes HepG2 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). HepG2 Transfection Reagent is supplied in liquid form at a concentration of 0.25 mg/ml, shipped at ambient temperature.

Description:
Cationic lipid based reagent is a proprietary formulation optimized for transfection of DNA and RNA into HepG2 cells.

Product Qualification:
HepG2 Transfection Reagent is tested functionally by transfection of HepG2 cells with a small interfering RNAs targeting 3 different genes (Lamin A/C, GAPDH, Cyclophilin B). Transfection Reagent is tested for absence of nuclease contamination and microbial contamination.

Storage:
Store reagent at 4ºC upon receipt. If stored properly, reagent is stable for 12 months.

Intended Use:
For *in vitro* use only.

Safety Data Sheet (SDS):
SDS documents are available online at altogen.com

***In Vivo* Transfection Kits (for compound testing in rodents):**
- Catalog #5010 / 5011 / 5012 - Lipid *In Vivo* Transfection Kit
- Catalog #5020 / 5021 / 5022 - Polymer *In Vivo* Transfection Kit
- Catalog #5030 / 5031 / 5032 - Nanoparticle *In Vivo* Transfection Kit
- Catalog #5040 / 5041 / 5042 - PEG-Liposome *In Vivo* Transfection Kit
- Catalog #5050 / 5051 / 5052 - Pancreas *In Vivo* Transfection Kit
- Catalog #5060 / 5061 / 5062 - Liver *In Vivo* Transfection Kit
- Catalog #5070 / 5071 / 5072 - Kidney *In Vivo* Transfection Kit

Transfection Controls and Recommended Products:
- Catalog #4060 - GFP-expressing plasmid DNA (25 ug)
- Catalog #4061 - Cell Cycle Arrest siRNA (5 nmol)
- Catalog #4062 - Apoptosis Inducing siRNA (5 nmol)

To Place an Order:
Both domestic (USA) and international orders can be placed online at altogen.com
Purchase Order (PO) should be e-mailed to orders@altogen.com

Transfection Resource: altogen.com/transfection-resource

Altogen Labs. GLP Compliant Pre-clinical CRO Laboratory Services: AltogenLabs.com

Recommended Transfection Protocols (<u>for 24-well plate</u>):

HepG2 Standard Transfection Protocol (24-well plate):	HepG2 Reverse Transfection Protocol (24-well plate):
1. Plate 7,500 - 12,000 HepG2 cells per well in 0.5 ml of complete growth medium 12–24 hours prior to transfection **2.** Wash with 1xPBS and add 0.5 ml of fresh growth medium **3.** Prepare transfection complexes by mixing 40 µl of serum-free medium, 5.5 µl of transfection reagent, and • 750 ng DNA (or mRNA), or • 30 nM - 50 nM of siRNA (or microRNA) **Referred to a final volume including growth medium* **4.** Incubate transfection complexes at RT for 15 - 30 minutes **5.** <u>Optional</u>: Add 2 µl of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity **6.** Add prepared transfection complexes to 0.5 ml of complete growth medium with HepG2 cells (from step 2) **7.** Incubate cells at 37ºC in a humidified CO_2 incubator **8.** Assay for phenotype or target gene expression 48 - 72 hours after transfection	**1.** Prepare HepG2 cell suspension: **a.** Trypsinize cells (0.05% Trypsin) for 3-5 minutes at 37°C **b.** Dilute in complete growth medium to 5 x 10^4 cells/ml **2.** Prepare transfection complexes by mixing 40 µl of serum-free medium, 5.5 µl of transfection reagent, and • 750 ng DNA (or mRNA), or • 30 nM - 50 nM of siRNA (or microRNA) **Referred to a final volume including growth medium* **3.** Incubate transfection complexes at RT for 15 - 30 minutes **4.** <u>Optional</u>: Add 2 µl of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity **5.** Plate 15,000 - 25,000 cells per well in 0.5 ml of complete growth medium (from step #1) into culture plate **6.** Add prepared transfection complexes (from step 3 or 4) **7.** Incubate cells at 37ºC in a humidified CO_2 incubator **8.** Assay for phenotype or target gene expression 48 - 72 hours after transfection
<u>Optional:</u> Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 µl of Transfection Enhancer reagent 12-24 hours after transfection	<u>Optional:</u> Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 µl of Transfection Enhancer reagent 12-24 hours after transfection
If the viability of HepG2 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant	If the viability of HepG2 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant

Scaling Up or Down Transfections:

Culture Vessel Surface Area (cm^2)	Volume of Growth Medium (ml)	Transfection Reagent (µl)	Complex Condenser (µl)	Transfection Enhancer (µl)
96-well, 0.3 cm x cm	0.12	1.5	0.3	0.3
24-well, 2 cm x cm	0.5	5.5	2	2
12-well, 4 cm x cm	1	12	4	4
6-well, 10 cm x cm	3	35	12	12
60-mm, 20 cm x cm	5	60	20	20
10-cm, 60 cm x cm	15	180	60	60

Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying HepG2 cell density and amount of transfection reagent. High passage of HepG2 cells and use of antibiotics (or growth factors) may require using larger volumes of HepG2 transfection reagent per reaction.

Certificate of Analysis:

Transfection reagent was tested *in vitro* and passed QC. Transfection reagent was tested for absence of nuclease contamination and microbial contamination. Rev. 04/30/2025.

Safety Data Sheet:

Please refer to <u>altogen.com</u> for Safety Data Sheet of the product.