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CACO2 Transfection Protocol (Kit for Colon Adenocarcinoma Cells, HTB-37)

Catalog No. 6347 Size: 0.5 ml
Catalog No. 6348 Size: 1.5 ml
Catalog No. 6349 Size: 8.0 ml

Contents and Shipping:
CACO2 Transfection Kit includes CACO2 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). CACO2 Transfection Reagent is supplied in liquid form at a concentration of 0.25 mg/ml, shipped at ambient temperature.

Description:
Polymer-based transfection reagent is a proprietary formulation optimized for transfection of DNA and RNA into CACO2 cells.

Product Qualification:
CACO2 Transfection Reagent is tested functionally by transfection of CACO2 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>):

CACO2 Standard Transfection Protocol (24-well plate):	CACO2 Reverse Transfection Protocol (24-well plate):
1. Plate 10,000 - 15,000 CACO2 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, 4.5 µl of transfection reagent, and • 500 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 CACO2 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 CACO2 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, 4.5 µl of transfection reagent, and • 500 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 20,000 - 30,000 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 CACO2 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 CACO2 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	1.2	0.3	0.3
24-well, 2 cm x cm	0.5	4.5	2	2
12-well, 4 cm x cm	1	10	4	4
6-well, 10 cm x cm	3	30	12	12
60-mm, 20 cm x cm	5	50	20	20
10-cm, 60 cm x cm	15	150	60	60

Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying CACO2 cell density and amount of transfection reagent. High passage of CACO2 cells and use of antibiotics (or growth factors) may require using larger volumes of CACO2 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. 04/29/2025.

Limited Use Label License:

The purchase of this product conveys to the purchaser the limited right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. This product is for research purposes only and is not for use in commercial applications of any kind. For information on obtaining additional rights, please contact Altogen Biosystems at <u>orders@altogen.com</u>.

Safety Data Sheet:

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

Limited Product Warranty:

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Altogen Biosystems. Altogen Biosystems shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product. This product is developed, manufactured and sold for research purposes only. Reagent is not suitable for administration to humans.