Altogen Biosystems

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PANC-1 Transfection Protocol (Kit for Non-endocrine Pancreatic Cancer)

Catalog No. 3225	Size: 0.5 ml
Catalog No. 3226	Size: 1.5 ml
Catalog No. 7076	Size: 8.0 ml

Contents and Shipping:

PANC-1 Transfection Kit includes PANC-1 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). PANC-1 Transfection Reagent is supplied in liquid form at a concentration of 0.3 mg/ml, shipped at ambient temperature.

Description:

Lipid-based proprietary formulation optimized for transfection of PANC-1 cell line.

Product Qualification:

PANC-1 Transfection Reagent is tested functionally by transfection of PANC-1 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 4°C 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 https://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 (<u>https://altogen.com</u>) using credit card payment. Purchase Order (PO) can be faxed at (702) 989-0841 (for USA only).

 Transfection Resource:
 altogen.com/transfection-resource

Altogen Labs. GLP Compliant Pre-clinical CRO Laboratory Services: <u>AltogenLabs.com</u>

Recommended Transfection Protocols (for 24-well plate):

PANC-1 Standard Transfection Protocol (24-well plate):	PANC-1 Reverse Transfection Protocol (24-well plate):		
1. Plate 10,000 - 15,000 PANC-1 cells per well in 0.5 ml of	1. Prepare PANC-1 cell suspension:		
complete growth medium 12–24 hours prior to transfection	a. Trypsinize cells (0.05% Trypsin) for 3-5 minutes at 37°C		
2. Wash with 1xPBS and add 0.5 ml of fresh growth medium	b. Dilute in complete growth medium to $5 \ge 10^4$ cells/ml		
3. Prepare transfection complexes by mixing 40 µl of serum-	2. Prepare transfection complexes by mixing 40 µl of serum-		
free medium, 5 μ l of transfection reagent, and	free medium, 5 µl of transfection reagent, and		
• 600 ng DNA (or mRNA), or	• 600 ng DNA (or mRNA), or		
• 30 nM - 50 nM of siRNA (or microRNA)	• 30 nM - 50 nM of siRNA (or microRNA)		
*Referred to a final volume including growth medium	* <i>Referred to a final volume including growth medium</i>		
4. Incubate transfection complexes at RT for 15 - 30 minutes	3. Incubate transfection complexes at RT for 15 - 30 minutes		
5. Optional: Add 2 µl of Complex Condenser. This reagent	4. Optional: Add 2 µl of Complex Condenser. This reagent		
reduces the size of transfection complex, therefore increasing	reduces the size of transfection complex, therefore increasing		
transfection efficiency; however it may increase cell toxicity	transfection efficiency; however it may increase cell toxicity		
6. Add prepared transfection complexes to 0.5 ml of	5. Plate 20,000 - 30,000 cells per well in 0.5 ml of complete		
complete growth medium with PANC-1 cells (from step 2)	growth medium (from step #1) into culture plate		
7. Incubate cells at 37° C in a humidified CO ₂ incubator	6. Add prepared transfection complexes (from step 3 or 4)		
8. Assay for phenotype or target gene expression 48 - 72	7. Incubate cells at 37° C in a humidified CO ₂ incubator		
hours after transfection	8. Assay for phenotype or target gene expression 48 - 72		
	hours after transfection		
Optional: Transfection efficiency can be increased by	Optional: Transfection efficiency can be increased by		
addition of Transfection Enhancer reagent. Add 2 µl of	addition of Transfection Enhancer reagent. Add 2 µl of		
Transfection Enhancer reagent 12-24 hours after transfection	Transfection Enhancer reagent 12-24 hours after transfection		
If the viability of PANC-1 cells being transfected is affected	If the viability of PANC-1 cells being transfected is affected		
at 16 - 24 hours post-transfection, the level of cytotoxicity	at 16 - 24 hours post-transfection, the level of cytotoxicity		
can be decreased by changing the growth medium and	can be decreased by changing the growth medium and		
eliminating redundant exposure of cells to transfectant	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	1.4	0.3	0.3
24-well, 2 cm x cm	0.5	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	160	60	60

Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying PANC-1 cell density and amount of transfection reagent. High passage of PANC-1 cells and use of antibiotics (or growth factors) may require using larger volumes of PANC-1 transfection reagent per reaction.

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>.

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