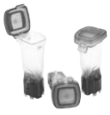
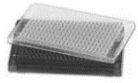
Nucleofection® Protocol for HCT 116 Cells – Transfection in Suspension

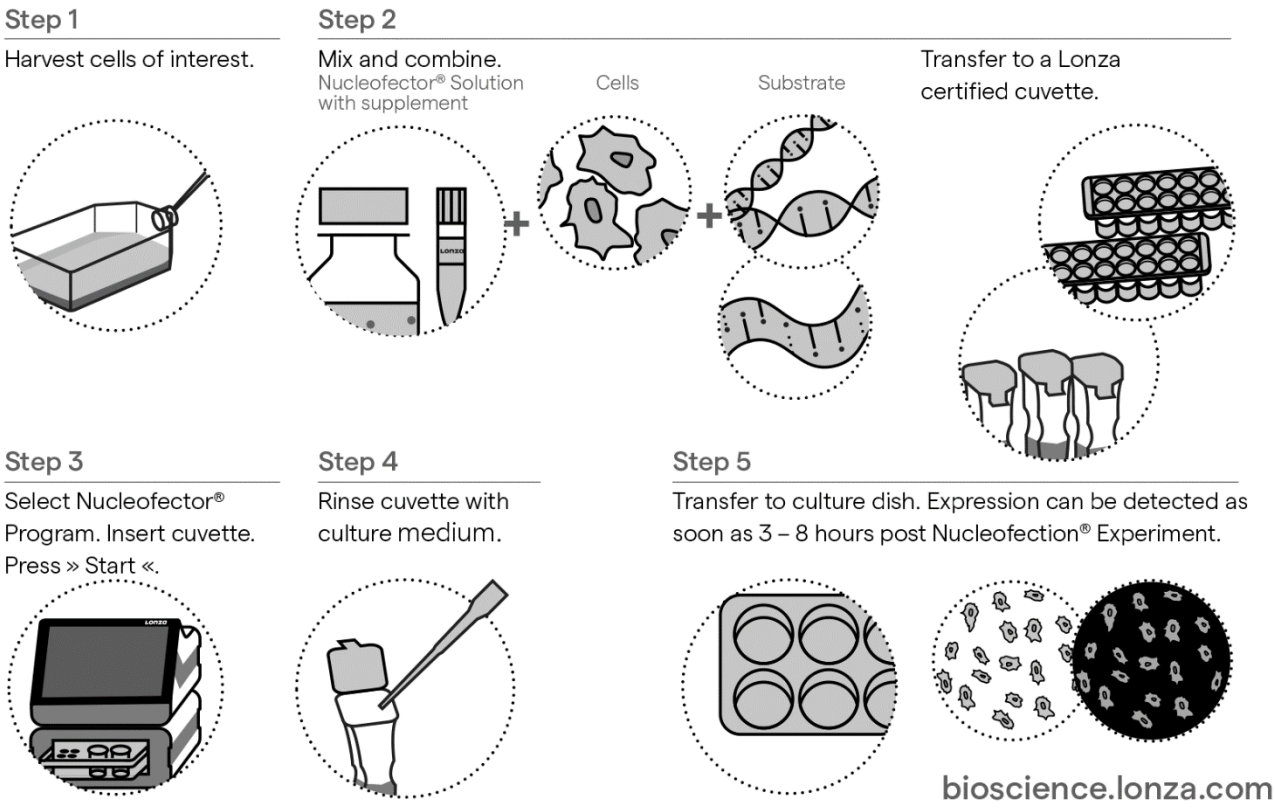
Transfection Efficiency and Viability

This Optimized Protocol describes how to transfect HCT 116 cells on the 4D-Nucleofector® System and the 384-well Nucleofector® System. Transfection efficiencies of up to 93% can be achieved using pulse code EN-113 and 0.4 µg of pmaxGFP™ Vector in 20 µL Nucleocuvette® Strips (analyzed 24h post Nucleofection® by flow cytometry). The cell viability of 62% was determined using the ViaLight® Plus Assay and normalized to untransfected control samples. Similar transfection efficiencies can be achieved with 100 µL, 20 µL, single-well, 16-well, 96-well, and 384-well Nucleofection® Vessels as it is possible to transfer the experimental settings between systems. Visit the **Lonza Knowledge Center** for more information, including citations.

				
Cat. No	**V4XC-1012/ V4XC-1024**	**V4XC-1032**	**V4SC-1096/ V4SC-1960**	**V5SC-1002*/ V5SC-1010***
Transfection volume	100 µL	20 µL	20 µL	20 µL
Size [reaction]	12/24	2 x 16	1 x 96/ 10 x 96	2 x 384/ 10 x 384
SE Cell Line Nucleofector® Solution	2 x 0.675 mL/ 2.25 mL	0.675 mL	2.25 mL/ 22.5 mL	22.5 mL/ 90 mL
Supplement 1	2 x 0.15 mL/ 0.5 mL	0.15 mL	0.5 mL/ 5 mL	5 mL/ 20 mL
pmaxGFP™ Vector (1 µg/µL in 10 mM Tris pH 8.0)	50 µg	50 µg	50 µg	50 µg/ 150 µg
Single Nucleocuvette® Vessel (100 µL)	12/24	-	-	-
16-well Nucleocuvette® Strips (20 µL)	-	2	-	-
96-well Nucleocuvette® Plate (20 µL)	-	-	1/10	-
384-well Nucleocuvette® Plate (20 µL)*	-	-	-	2/10
To be used in conjunction with	4D-Nucleofector® Core and X Unit	4D-Nucleofector® Core and X Unit	4D-Nucleofector® Core and 96-well Unit	384-well Nucleofector® System

* 384-well Nucleocuvette® plates are best handled with a liquid handling system. Contact **Scientific Support** for further information.

Nucleofection® Handling



Recommended Kits: SE Cell Line Nucleofector® Kits

The SE Cell Line Kits are used with the modular 4D-Nucleofector® System or the high-throughput 384-well Nucleofector® System for efficient transfection of various mammalian cells including HCT 116 cells. The kits consist of two components that are not sold individually (conductive polymer cuvettes and a solution box) and are available in a single, 16-well, 96-well, and 384-well Nucleocuvette® Format.
SE Cell Line Kit components are listed in the table above.

Storage and Stability

Nucleofector® SE Cell Line Kits are shipped at room temperature. After receiving the kits, store Nucleofector® Solution, Supplement, and pmaxGFP™ Vector at 2–8°C and Nucleocuvette® Vessels at room temperature. For long-term storage, pmaxGFP™ Vector is ideally stored at -20°C. The expiration date is printed on the solution box.

Once the Nucleofector® Supplement is added to the Nucleofector® Solution, it is stable for three months at 4°C.

Required Material

- Respective Nucleofector® System
- Supplemented SE Cell Line Nucleofector® Solution at room temperature

Note
Please make sure to add Supplement 1 to the Nucleofector® Solution. The ratio of Nucleofector® Solution to Supplement is 4.5:1 (see Table 1). Prepare enough supplemented solution for the number of samples you want to transfect (see Table 3).

- Respective Nucleocuvette® Vessel
- If working with 20 µL Nucleocuvette® Strips or Plates: Use suitable pipette tips. Please make sure that your pipette tips reach the bottom of the Nucleocuvette® Wells without getting stuck

- If working with 384-well or 96-well Nucleocuvette® Plates: The 384-well Nucleofector® System and the 4D-Nucleofector® 96-Well Unit can be used either manually or automated on standard liquid handling instruments. For automated processing, consumables specific to your respective liquid handling system are required. For manual processing, we recommend using a multi-channel pipette
- Substrate of interest, highly purified, preferably by using endotoxin-free kits; for plasmid DNA A260/A280 ratio should be ~1.8
- Supplied pmaxGFP™ Vector, stock solution 1 µg/µL

Note
When using pmaxGFP™ as a positive control, dilute the stock solution to an appropriate working concentration. Further details are provided in Table 3. The volume of substrate solution added to each sample should not exceed 10% of the total reaction volume (2 µL for 20 µL reactions; 10 µL for 100 µL reactions).

- Cell culture plates of your choice
- For trypsinization: 0.5 mg/mL trypsin and 0.2 mg/mL EDTA in PBS (5x concentrated) and supplemented culture media or PBS with 0.5% BSA
- Culture medium: McCoy's 5A medium supplemented with 10% fetal calf serum (FCS), 100 µg/mL streptomycin, 100 U/mL penicillin, 2 mM glutamine. Prewarm appropriate volume of culture medium to 37°C (see Table 2)
- Recovery medium (optional): Low calcium medium, e.g. RPMI
- Appropriate number of cells/sample (see Table 2)

Pre Nucleofection®

Cell Culture Recommendations
The viability and overall health of cells prior to transfection is well known to be important for optimal transfection results. Prior to transfection, cells should be at least 90% viable and have had adequate time to recover from passaging. We recommend the following culture conditions for HCT 116 cells:

- Replace media every 2–3 days
- Passage cells 3 times per week
- A subcultivation ratio 1:3–1:8 is recommended
- Use early passages for Nucleofection® Experiments
- Subculture cells 2 days before Nucleofection® Experiment
- Optimal confluency for Nucleofection® Experiments: 80–90%. Higher cell densities may cause lower Nucleofection® Efficiencies

Trypsinization
- Remove media from the cultured cells and wash cells once with PBS; use at least same volume of PBS as culture medium
- For harvesting, incubate the cells at 37°C with 0.5 mg/mL trypsin and 0.2 mg/mL EDTA
- Inactivate trypsinization reaction with supplemented culture medium or PBS with 0.5% BSA

Nucleofection®

Table 3 shows the recommended amount of substrate, cell number, and Nucleofection® Pulse Code for each sample.

- Please make sure that Supplement 1 has been added to the required amount of Nucleofector® SE Cell Line Solution (see Table 1)
- Start your respective Nucleofector® System and create or upload the experimental parameter file (see system manual for details)
- Select/Check for the appropriate Nucleofector® Pulse Code (see Table 3), we recommend pulse code EN-113 for the X Unit or corresponding programs for other units
- Prepare cell culture plates by filling an appropriate number of wells with the recommended volume of culture media (see Table 4) and pre-incubate (equilibrate) the plates at 37°C, 5% CO_2
- Prewarm an aliquot of culture medium to 37°C (see Table 4)
- Make sure to dilute your substrate to the recommended working concentration (see Table 3)
- Harvest the cells by trypsinization (see Trypsinization)
- Take an aliquot of the cell suspension and count cells to determine the cell density

- Transfer the required amount of cells (see Table 3) to a new tube and pellet by centrifugation at 90xg for 10 minutes at room temperature. Gently remove all supernatant. Note that cell pellet may be looser than normal.
- Resuspend the cell pellet carefully at room temperature in supplemented Nucleofector® SE Cell Line Solution (see Table 3). Gently pipette the cells to obtain homogeneous cell suspension.
- Prepare master mixes by dividing cell suspension according to the number of substrates
- Add required amount of substrates to each aliquot (max. 10% of final sample volume)

Note

When working with RNA, it is recommended to avoid longer incubation times as RNA is susceptible to degradation, although Nucleofector® Solutions are tested for absence of RNase activity

- Transfer master mixes into the respective Nucleocuvette® Vessels

Note

It is important to work as quickly as possible as leaving cells in Nucleofector® Solution for extended periods of time may lead to reduced transfection efficiency and viability. Avoid air bubbles while pipetting. When working in a 96- or 384-well format it is recommended to either pre-dispense each cell suspension into a sterile round-bottom 96-well plate or to use reservoirs for multichannel pipettes.

- Gently tap the Nucleocuvette® Vessels on the benchtop to make sure the sample covers the bottom of the reaction vessel

Note

When using the 384-well Nucleocuvette® Plate, instead of tapping you may briefly shake it with an appropriate microtiter-plate shaker to make sure the sample covers the bottom and sides of the wells without air bubbles.

- Place Nucleocuvette® Vessel with closed lid into the retainer of the Nucleofector® System. Check for proper orientation of the Nucleocuvette® Vessel
- Start the Nucleofection® Process by pressing “Start” on the display of your Nucleofector® System (for details, please refer to the respective system manual)
- After the Nucleofection® Process is completed, carefully remove the Nucleocuvette® Vessel from the retainer
- **Optional:** A recovery step can help to improve viability after the Nucleofection® Experiment. Add 100–300 µL pre-equilibrated recovery medium to the cuvette (instead of the standard culture media) and gently transfer it to a reaction tube. Place the cell suspension in an incubator for 5–10 minutes. Transfer the sample into the prepared culture dish and continue with section post Nucleofection®
- Resuspend cells with prewarmed medium (for recommended volumes see Table 4). Mix cells by gently pipetting up and down two to three times. When working with the 100 µL Nucleocuvette® Vessels, use the supplied pipettes and avoid repeated aspiration of the sample
- Plate desired amount of cells in culture system of your choice (for recommended volumes, see Table 4)

Post Nucleofection®

- Incubate the cells in humidified 5% CO_2 incubator at 37°C until analysis. Gene expression or downregulation, respectively, is often detected after just 4–8 hours.

Selected References

- Wienert, Beeke et al. “Timed inhibition of CDC7 increases CRISPR-Cas9 mediated templated repair.” Nature communications (2020) vol. 11(1): 2109. doi:10.1038/s41467-020-15845-1
- Hassin, Ori et al. “Different hotspot p53 mutants exert distinct phenotypes and predict outcome of colorectal cancer patients.” Nature communications (2022) vol. 13(1): 2800. doi:10.1038/s41467- 022-30481-7
- Hand, Travis H et al. “Catalytically enhanced Cas9 through directed protein evolution.” The CRISPR journal (2021) vol. 4(2): 223-232. doi:10.1089/crispr.2020.0092
- El Khattabi, Laila et al. “A pliable mediator acts as a functional rather than an architectural bridge between promoters and enhancers.” Cell (2019) vol. 178(5): 1145-1158.e20. doi:10.1016/j.cell.2019.07.011

Table 1: Volumes required for a single reaction

	100 µL	20 µL
Volume of Nucleofector® Solution	82 µL	16.4 µL
Volume of Supplement 1	18 µL	3.6 µL

Table 2: Required amounts of cells and media for a Nucleofection® Reaction

		16-well / 96-well	384-well
Reaction volume	100 µL	20 µL	20 µL
Culture medium per sample post Nucleofection® (for transfer and culture)	1.4 mL	255 µL	95 µL
Cell number per Nucleofection® Sample	1 x 10^6 (Lower or higher cell numbers may influence transfection results)	2 x 10^5 (Lower or higher cell numbers may influence transfection results)	2 x 10^5 (Lower or higher cell numbers may influence transfection results)

Table 3: Contents of one Nucleofection® Sample and recommended Pulse Code

		Single Nucleocuvette® Vessel	16-well Nucleocuvette® Strips	96-well Nucleocuvette® Plate	384-well Nucleocuvette® Plate
Reaction volume		100 µL	20 µL	20 µL	20 µL
Cells		5–10 x 10^5	2 x 10^5	2 x 10^5	2 x 10^5
Substrate*	pmaxGFP™ Vector	2 µg	0.4 µg	0.4 µg	0.4 µg
or	plasmid DNA (in H_2O or TE)	1–5 µg	0.2–1 µg	0.2–1 µg	0.2–1 µg
or	siRNA	30–300 nM siRNA (3–30 pmol/sample)	30–300 nM siRNA (0.6–6 pmol/sample)	30–300 nM siRNA (0.6–6 pmol/sample)	30–300 nM siRNA (0.6–6 pmol/sample)
or	mRNA	The amount of RNA must be optimized for each new mRNA. Too much RNA can have toxic effects, while too little RNA can lead to poor expression. RNA characteristics such as size or RNA features (e.g. poly-A tail, cap, or IRES) can have a significant impact on translation.			
or	RNPs	A dose-respond experiment should be performed to find the ideal RNP concentration after an appropriate RNP molar ratio has been established			
SE Cell Line Solution		100 µL	20 µL	20 µL	20 µL
Pulse Code		EN-113	EN-113	EN-113	EN-113-AA

* Volume of the substrate should comprise a maximum of 10% of the total reaction volume

Table 4: Culture volumes recommended for post Nucleofection® Steps and sample transfer

		16-well / 96-well*	384-well**
Reaction volume	100 µL	20 µL	20 µL
Culture medium pre-filled in 6-well culture plate	1 mL	-	-
Culture medium pre-filled in 96-well culture plate	-	175 µL	-
Culture medium pre-filled in 384-well culture plate	-	-	55 µL
Culture medium to be added to the sample post Nucleofection®	400 µL	80 µL	40 µL
Volume of sample transferred to culture plate	complete sample (use supplied pipettes)	25 µL	5 µL

* Maximum well volume: 200 µL. ** Maximum well volume: 60 µL

Additional Information

For additional information and an up-to-date list of Nucleofector® References, please visit the Lonza Knowledge Center:
https://knowledge.lonza.com

For more technical assistance, contact our Scientific Support Team:

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