



Demeetra – CHO-K1 Suspension Transfection Protocol

For commercial purposes: gRNA's can be ordered as 2-part gRNA's (crRNA and tracrRNA) from IDT which can be complexed at 200uM or resuspended @ approximately 5 ug/ul in Duplex buffer (cat# 1072570).

For Research purposes: gRNA's can be ordered as single synthetic guides through Synthego or IDT in which Demeetra recommends resuspending in Nuclease-free water or Duplex buffer (cat# 1072570, IDT) @ approximately 5 ug/ul.

- We recommend keeping the gRNA's at a high concentration in order to keep the transfection volumes and ratios to a minimum. **Working concentration is 1 ug/ul in Buffer R.**

Prepare Suspension CHO-K1 cells for NEON NxT Electroporation:

- Cultivate the required number of cells by seeding a flask containing fresh growth medium 1-2 days prior to electroporation.

Note* Cell viability should be >90%

- o The preferred range for 10 μ L NEON NxT tip \rightarrow 2.0×10^5 to 2.5×10^5 cells
- o The preferred range for 100 μ L NEON NxT tip \rightarrow 1.0×10^6 to 2×10^6 cells
- Pre-warm culture media
- Count cells to determine the cell density of the suspension. Add 20 μ L of cell suspension to 20 μ L 0.04% Trypan Blue solution, mix by pipetting up and down 5-7 times.
- Transfer cells (number of cells per transfection) to a 1.5 mL reaction tube or 15 mL falcon tube and centrifuge the cells at 250 x g for 5 minutes at room temperature.
- Resuspend the cell pellet by pipetting up and down 5-7 times in Resuspension Buffer R considering the range information for 10 μ L or 100 μ L Neon NxT tip
- After transfecting, immediately place the cells in a 24-well plate containing the corresponding culture media **WITHOUT antibiotics.**

Note* Avoid clumps in the cells culture before transfecting.

Note* Avoid storing cells in Resuspension Buffer R for more than 15 minutes at room temperature.

- o 48-well \rightarrow 10 μ L NxT tip, $2.0 - 2.5 \times 10^5$ cells (suspension)
- o 24-well \rightarrow 10 μ L NxT tip, $2.0 - 2.5 \times 10^5$ cells (suspension)
- Demeetra recommends aliquoting out cells into separate tubes according to volumes of protein, mRNA, gRNAs, and knock-in cargo (if any).

Note* Keep volume of gene editing reagents to a minimum in order to maintain consistent transfection conditions (10% of total volume).

Note* If using Cas-CLOVER mRNA, there is no precomplexing step.

Note* Cell Number accounts for extra volume of the reaction which helps to avoid air bubbles during electroporation.



- See below table for typical reaction set ups for 10 μL NxT tip.

	NTC	CC Rxn #1 (1:2)	CC Rxn #2 (1:4)
mRNA	1 μL eGFP	1 μL CC	1 μL CC
gRNA (1 $\mu\text{g}/\mu\text{L}$)	-	1 μL each	2 μL each
PBS	3 μL	1 μL	-
Cells	2.6×10^5	2.6×10^5	2.6×10^5
Cell Volume	9 μL	9 μL	8 μL
Total Volume	13 μL	13 μL	13 μL

- Once all components have been aliquoted in their respective tubes, mix all contents by pipetting up and down 3-4 times.
- Fill wells with pre-warmed culture medium **WITHOUT antibiotics** if not done so already.
 - o Recommended volumes
 - 24-well \rightarrow 500 μL

Set up NEON NxT Pipette Station according to manufacturer's instructions (allow Buffers to reach RT prior to transfection)

- Buffer E10/E for 10 μL NxT tips \rightarrow 2 mL for NxT Tubes or 3 mL for old version
- Buffer E100/E2 for 100 μL NxT tips \rightarrow 2 mL for NxT Tubes or 3 mL for old version

Load the Pipette according to manufacturer's instructions

- Press the plunger on the NEON NxT Pipette to the first stop and immerse the tip into the cell-payload mixture previously prepared (see table). Slowly release the plunger to aspirate the cell-payload mixture into the NEON NxT Tip.
 - o 10 μL volume is a total of 2.0×10^5 cells per transfection (or well)
 - o Avoid air bubbles as they will cause arcing during electroporation
- Dock the NEON NxT Pipette with the sample vertically into the NEON NxT Tube placed in the NEON NxT Pipette Station until you hear a click sound. Ensure that the pipette projection is inserted into the groove of the pipette station, and that the tip is submerged in electrolytic buffer.

Run electroporation protocol

- For CHO-K1 cells,
 - o Run program \rightarrow **1,650V, 10ms, 3 pulses for 10 μL tip**

- After successful electroporation, directly add content from NxT tip to the culture plate

Allow cells to recover for 48 to 72 hours, while monitoring transfection efficiencies and/or cell viabilities.

Note* Cell viability greatly influences downstream workflows. Cultures should be >70% viable after recovery.

Begin any downstream analyses after 48 to 72 hours or let cells recover for 5 days then begin single-cell cloning.

Specific downstream workflows include:

- Total DNA or gDNA extraction and purification
- T7 EnGen Mutation Detection Kit (NEB E3321S) for gene editing efficiency
- TOPO Cloning kit (NEB E1202S or any other equivalent TOPO cloning kit)
- Next generation sequencing or sanger sequencing
- Synthego ICE CRISPR Analysis Tool