



Demeetra – CHO-K1 Super PiggyBac → Suspension

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%

- The preferred range for 10ul NEON NxT tip → 2.0×10^5 to 2.5×10^5 cells
- The preferred range for 100ul NEON NxT tip → 1.0×10^6 to 5×10^6 cells
- Pre-warm culture media and aliquot into plates, keep in incubator until transfection.
- Count cells to determine the cell density of the suspension. Add 20ul of cell suspension to 20ul 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 5-7 times in Resuspension Buffer R considering the range information for 10 uL or 100 uL Neon NxT tip
- After transfecting, immediately place the cells in a 24-well plate containing the corresponding culture media **WITHOUT antibiotics.**

Note* Avoid storing cells in Resuspension Buffer R for more than 10 minutes at room temperature.

- 48-well → 10uL NxT tip, $2.0 - 2.5 \times 10^5$ cells (suspension)
- 24-well → 10uL NxT tip, $2.0 - 2.5 \times 10^5$ cells (suspension)
- Demeetra recommends aliquoting cells into separate tubes according to volumes of mRNA and transposon cargo.

Note* Keep volume of Super PiggyBac reagents to a minimum to maintain consistent transfection conditions (10% of total volume). Can dilute transposon in Buffer R if necessary.

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

- See below table for typical reaction Super PiggyBac set ups for 10 uL NxT tip.

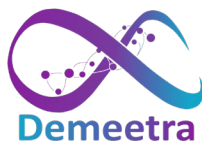
Recommended Condition

	NTC	Ex.1 (1:1)	Ex.2 (2:1)	Ex.3 (4:1)	Ex.4 (4:1)
Transposase	1ul eGFP mRNA	0.5ul (500ng) Super PB mRNA	0.5ul (500ng) Super PB mRNA	0.25ul (250ng) Super PB mRNA	0.125ul (125ng) Super PB mRNA
Transposon	-	500ng (Xul)	1ug (Xul)	1ug (Xul)	500ng (Xul)
PBS/Buffer R	3ul	X ul to 4ul	X ul to 4ul	X ul to 4ul	X ul to 4ul
Cells	3.0×10^5	3.0×10^5	3.0×10^5	3.0×10^5	3.0×10^5
Cell Volume	8ul	8ul	8ul	8ul	8ul
Total Volume	12ul	12ul	12ul	12ul	12ul

- Once all components have been aliquoted in their respective tubes, pipet up and down 3-5 times to mix and fill wells with pre-warmed culture medium and supplements **WITHOUT antibiotics** if not done so already.
 - Recommended volumes
 - 24-well → 500uL
 - 48-well → 250uL

Set up NEON NxT Pipette Station according to manufacturer's instructions (buffer should be at RT)

- Buffer E10/E for 10 uL NxT tips → 2 mL for NxT Tubes or 3 mL for old version



- Buffer E100/E2 for 100 uL NxT tips → 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 10uL volume is a total of 2.5×10^5 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 → **1,650V, 10ms, 3 pulses for 10uL tip**
- After successful electroporation, directly add content from NxT tip to the culture plate

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

Note* Cell viability greatly influences downstream workflows.

After 72 hours recovery, check viability, and if viability is >90% proceed with antibiotic/ glutamine selection to enrich integration-positive pool of cells for single cells cloning.

Specific downstream workflows include:

- Total DNA or gDNA extraction and purification
- rtPCR
- Splinkerette PCR
- Next generation sequencing or sanger sequencing