



Demeetra – CHO-K1 Harbor-IN → Lipofectamine MessengerMAX

Prepare CHO-K1 cells for Lipofectamine transfection:

- Cultivate the required number of cells by seeding a flask containing fresh growth medium 1-2 days prior to transfection.
- Adherent cells should be 70%-90% confluent on the day of transfection by seeding a plate containing fresh growth medium 1-2 days prior to transfection. Suspension cells can be processed just before transfection and the cell number range is listed after adherent numbers.
 - o The range for 24-well plate → 0.5×10^5 to 2×10^5 cells, 1×10^5 to 2.5×10^5 cells
 - o The range for 12-well plate → 1.0×10^5 to 4×10^5 cells, 2×10^5 to 6×10^5 cells
 - o The range for 6-well plate → 0.25×10^5 to 1×10^6 cells, 5×10^5 to 1×10^6 cells
- Pre-warm culture media, PBS (without Ca^{2+} and Mg^{2+}), and Opti-MEM to 37°C.

Note* Cell viability should be >90%

- 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 and centrifuge the cells at 250 x g for 5 minutes at room temperature.
- Wash cells with PBS and centrifuge as above
- Aspirate the PBS and resuspend the cell pellet thoroughly in normal growth media
- After resuspension, immediately place the cells in a 24-well plate containing the corresponding culture media (preferred without antibiotics).

Note* Avoid clumps in the cells culture before transfecting.

Note* Keep volume of Harbor-IN reagents to a minimum to maintain consistent transfection conditions (10% of total volume).

- See below table for typical 12-well set up for Lipofectamine MessengerMAX. **Recommended Conditions.**
- One Master Mix tube may be used for Opti-MEM and Lipofectamine MessengerMAX reagent
 - o 24-well plate → 1 uL Lipo Reagent + 25 uL Opti-MEM per well
 - o 12-well plate → 2.5 uL Lipo Reagent + 75 uL Opti-MEM per well
 - o 6-well plate → 5 uL Lipo Reagent + 125 uL Opti-MEM per well
- **Incubate diluted MessengerMAX Reagent in Opti-MEM for 10 minutes at Room Temp.**

	NTC	Ex.1 (1:1)	Ex.2 (2:1)	Ex.3 (2:1)	Ex.4 (4:1)
Transposase	1ul eGFP mRNA	0.5ul (0.5ug) Harbor-IN mRNA	0.5ul (0.5ug) Harbor-IN mRNA	1ul (1ug) Harbor-IN mRNA	0.5ul (0.5ug) Harbor-IN mRNA
Transposon	-	0.5ug (Xul)	1ug (Xul)	2ug (Xul)	2ug (Xul)
PBS	3ul	X ul to 5ul	X ul to 5ul	X ul to 5ul	X ul to 5ul
Opti-MEM	75ul	75ul	75ul	75ul	75ul
Total Volume	80ul	80ul	80ul	80ul	80ul

- Once all components have been aliquoted in their respective tubes, add Master Mix of diluted Lipofectamine MessengerMAX Reagent to the tubes and mix well by flicking the tube or pipetting up and down 5-10 times.
- **Incubate for 5 minutes at Room Temperature.**
- Add the mRNA-lipid complex to cells dropwise to each respective well/plate



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

Begin any downstream analyses after 48-to-72-hour recovery or let cells recover for 5 days then begin gradual/stepwise 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