HCT-116 VIM RFP (ATCC® CCL-247EMT™) epithelial-mesenchymal transition (EMT) induction instruction

Materials:

1. HCT-116 VIM RFP cells (ATCC® CCL-247EMT™)
2. McCoy's 5A Medium (ATCC® 30-2007™)
3. Fetal Bovine Serum (FBS) (ATCC® 30-2020™)
4. Trypsin-EDTA Solution (1X) (ATCC® 30-2101™)
5. DPBS (ATCC® 30-2200™)
6. Anti-miR-200 LNA (Qiagen #339160 YFI 0450012)
7. Opti-MEM (Thermal Fisher #31985062)
8. RNAiMAX (Thermal Fisher #13778030)

Instructions:

A. The following directions are for a 12 well plate. They can be scaled up or down, accordingly.

Oligo stock should be re-suspended at a final concentration of 50 µM.

B. Culture cells for induction: Thaw one vial of HCT-116 VIM RFP cells and plate the cells in T-75 flask. Cell cultures generally reach 70-80% confluent in about 2-3 days.

C. Induction protocol:

1. Prepare HCT-116 VIM RFP (ATCC® CCL-247EMT ™) cells for induction
   1.1 Warm culture medium (McCoy's 5A with 10% FBS) to 37 ^{O}C.
   1.2 Harvest HCT-116 VIM RFP cells using Trypsin-EDTA solution.
   1.3 Resuspend the cells in warmed culture medium. Centrifuge the cell suspension @ approximately 250 X g for 5 minutes. Aspirate the liquid.
   1.4 Gently resuspend the cell pellet in warmed culture media and count viable cells using Trypan blue.
   1.5 Reuspend cells at a density of 2.0 x 10^5 cells/mL and put the cells in a 37°C + 5% CO_2 incubator.

2. Prepare siRNA transfection complexes:
   2.1 Bring RNAiMAX and Opti-MEM to room temperature.
   2.2 Label two sterile microcentrifuge tubes (–CONTROL, and +LNA) and pipette 100 µl Opti- MEM to each of them.
   2.3 Add 3 µL of LNA to '+LNA' tube and 3 µL of DPBS to '–CONTROL' tube. Gently mix.
   2.4 Add 3 µL of RNAiMAX to each tube. Mix thoroughly.
   2.5 Incubate transfection complexes at room temperature for 10 minutes.

3. Mix transfection complexes with cells
   3.1 Transfer the prepared '–CONTROL' and '+LNA' transfection complexes to wells of a 12-well plate.
   3.2 Mix cell suspension and add 500 µl of the cell suspension to the appropriate wells.

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3.3 Add an additional 500 µL of culture media to each well.
3.4 Gently rock plate back and forth at least 10 times.
3.5 Put the cells into a 37°C with 5% CO_2 incubator to complete the 1st induction.

4. When the cells grow to approximately 70-80% confluent (usually 3-4 days), repeat step 1 to 3 to complete the 2nd induction (Note: The number of live cells and the cell growth rate may decline due to toxicity of LNA to the cells).

5. When the cells grow to approximately 60-80% confluent (usually ~4 days), perform the 3rd induction. The 3rd induction uses half dose of LNA (100 µL Opti-MEM + 1.5 µL of LNA or DPBS + 1.5 µL of RNAiMAX).

6. When the cells reach approximately 60-80% confluent, repeat step 5 to perform 4th induction cycle, and so on. Generally, RFP expression can be observed after the 6th induction.

Reference for preparing siRNA transfection complexes:

	-CONTROL	+LNA
Opti-MEM media	100 µL	100 µL
Oligos		3 µL or 1.5 µL
DPBS	3 µL or 1.5 µL	
RNAiMAX	3 µL or 1.5 µL	3 µL or 1.5 µL
Cell Suspension	500 µL	500 µL
Culture Media	500 µL	500 µL

Reference:

Park SM, *et al*. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* 22: 894-907, 2008. PubMed: 18381893