miR-Tuners Set of 3 Control Vectors

Store at -20°C

Cat. No.	Description	Quantity
LV104	miR-Tuners Set of 3 Control Vectors	3 x 10 µg

Product Description

miR-Tuners are short, synthetic miRNA response element sequences which reside in the 3' UTR of the gene of interest (GOI). These sequences are responsive to a native miRNA species which is widely expressed in many human and mouse cells. Using the miR-Tuner Low, Med or Hi will allow for precise low, medium or high expression levels, respectively, of the GOI in mammalian cells. The miR-Tuner element is cloned within the 3'UTR of the reporter gene Firefly Luciferase, which allows for simple validation of the miR-Tuner system by measuring and comparing output luminescence.

Kit Components

Component	Concentration	Part No.
pLenti-PGK-Luciferase-miR-Tuner-Low	500 ng/µl	LV104-Low
pLenti-PGK-Luciferase-miR-Tuner-Med	500 ng/µl	LV104-Med
pLenti-PGK-Luciferase-miR-Tuner-Hi	500 ng/µl	LV104-Hi

Additional Materials Required

Material	Recommended Product	Cat. No.
Mammalian cell line (e.g. HEK293T)	ProAdhere 293T Cells	LV592
Growth medium	Prigrow III Medium	TM003
Fetal Bovine Serum (FBS)	USDA Research Grade Origin Fetal Bovine Serum	TM999-100
Cell culture 6-well plates	6-Well Multiwell Cell Plates	P0100
Transfection reagent	DNAfectinTM Plus Transfection Reagent	G2500
GFP expressing vector	pLenti-CMV-GFP-2A-Puro-Blank Vector	LV590
Phosphate buffered saline (PBS)	-	-
Luciferase Assay Kit	Luciferase Assay Kit	G287
White opaque 96-well plate	-	-
Multimode plate reader or luminometer	-	-

Storage

1 year (when at -20°C or below in a non-frost free freezer).

Protocol

The following protocol is provided as a general guideline only and is to be used as a starting point for determining optimal conditions for target cell transfection.

1. In a 6-well cell culture plate seed the cell line into five out of the six wells. Grow the cell line until it reaches 70-80% confluence at 37°C in a CO_2 incubator.
2. Prepare transfection samples:
    a. LV104-Low
    b. LV104-Med
    c. LV104-Hi
    d. LV590 (Control)
    e. No DNA (Control)
3. For each transfection sample, prepare the complexes as follows:
    - Solution A: Dilute 3.0 µg of DNA into 100 µl of serum-free, antibiotic-free medium.
    - Solution B: Vortex DNAfectin™2100 thoroughly prior use, then dilute 10-20 µl of DNAfectin™2100 in 100 µl serum-free, antibiotic-free medium.
    - Incubate Solution A and B at room temperature for 5 min.
4. Combine the solutions, mix gently to ensure uniform distribution and incubate for 20 min at room temperature. Complexes are stable at room temperature for 3-5 h.
5. Add 0.8 ml of serum-free, antibiotic-free medium to each DNAfectin™2100-DNA complex. Mix solution gently.
6. Remove growth medium from the cells and add the total DNAfectin™2100-DNA solution to each well containing cells.
7. After 5-8 h, remove transfection solution from the cells and add 2.0 ml of growth medium (with serum and antibiotics). Incubate the cells at 37˚C in a CO_2 incubator for a total of 18-24 h.
8. 24 h after transfection, inspect the LV590 (Control) well for GFP fluorescence. If the transfection was successful, >60% of cells should display bright GFP fluorescence under a fluorescence microscope and you may proceed with the protocol. If very few cells display GFP fluorescence, the transfection was unsuccessful and the protocol must be repeated and/or optimized from the beginning.
9. Remove the media from LV104-Low, LV104-Med, LV104-Hi and No DNA (Control) wells.
10. Gently wash the cells with phosphate buffered saline (PBS), then aspirate.
11. Add 150 µl of Cell Lysis Buffer (Cat. No. G287-2 from the Luciferase Assay Kit) to each well. Using an orbital shaker, gently shake at room temperature for 20 min.
12. Pipette up and down to loosen adhered cells and lysate, and then transfer each lysate into a labelled 1.5 ml tube chilled on ice.
13. Centrifuge the 1.5 ml tubes for 1 min at 12,000 rpm to clear the lysate, place back on ice.
14. Transfer 20 µl of each cell lysate sample in triplicate into a 96-well white, opaque microplate.
15. Quickly add 20 µl of Luciferase Assay Reagent (Cat. No. G287-1 from the Luciferase Assay Kit) to each well and mix by pipette.
16. Immediately read luminescence using a multi-mode plate reader or luminometer equivalent.

Assessing Results

The relative luminescence units (RLU) is directly correlated to the gene expression levels of Firefly Luciferase controlled by miR-Tuners. Output RLU should follow the trend of lowest to highest as follows: No DNA (Control), LV104-Low, LV104-Med, LV104-Hi. If desired, the output RLU from the samples can be normalized using the No DNA (Control) by averaging these wells and subtracting this value from the RLU of the samples.

Applied Biological Materials Inc. • 1-866-757-2414 • info@abmGood.com • www.abmGood.com