EndoGenius Inducer Assay

50 rxn, 100 rxn

Cat No: *EGI*50, *EGI*100

Shipping : Ship at ambient temperature.
Storage : See Assay Components section

I. Assay Components

Components	EGI50 50 Assays	EGI100 100 Assays	Storage Conditions
Active Mix	55 µl	110 µl	Store at -20°C. Avoid repeated freeze and thaw.
Control Mix	55 µl	110 µl	Store at -20°C. Avoid repeated freeze and thaw.
2x Reaction Buffer	120 µl	240 µl	Store at 4-8°C.
Dilution Buffer	16 ml	32 ml	Store at 4-8°C.
Encapsulation Buffer	55 µl	110 µl	Store at 4-8°C.

II. General Information

Important implications for human health have been made from what has been learned from overexpression studies, and these implications have changed our understanding of the causes and treatment of diseases. Since gene transfer vectors generally have a limited insert size, therapeutic gene entry is often limited to the intracellular delivery of a splice variant of a gene. However, for the proper regulation of cellular processes, it can be extremely important that all splice variants of a gene expressed in the cell of interest are expressed at the correct rate. Alternative splice is an important phenomenon in nature and at least one-third of human genes are thought to be subject to alternative splice processing (1).

Induction of endogenous gene expression using specific *EndoGenius* Inducer Assay results in expression of all splice variants that is expressed in that specific cell or tissue. The importance of correct stoichiometric expression of all splice variants of a gene has been demonstrated for angiogenesis in a mouse model. It has been shown that induction of endogenous gene expression of VEGF-A results in the formation of more mature vessels compared to exogenous introduction of the gene encoding only one splice variant of VEGF-A (2).

EndoGenius Inducer Assay allows both overexpression of even the largest gene in the genome (Figure 1) and overexpression of all cell-specific expressed variants.

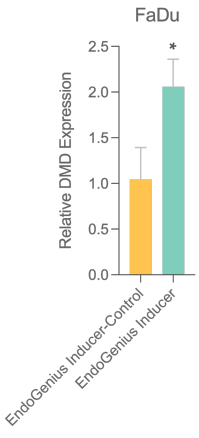

Figure 1. Relative expression of DMD, the largest gene in the human genome, when induced with specific *EndoGenius* Inducer Assay *in vitro*.

Utilization of *EndoGenius* Inducer Assay allows specific gene overexpression (Figure 2A) with minimal off-target effects (Figure 2B). It is quite easy to carry out an overexpression assay to see functional effects of overexpressing an endogenous gene. For example, overexpression of a specific oncogene results in increase in viability (Figure 2C).

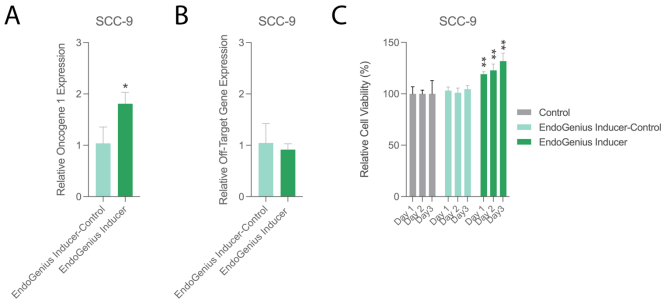

Figure 2. A. *EndoGenius* Inducer specifically induce significantly overexpression of Oncogene 1, B. with no alteration in other genes. C. Overexpression of Oncogene 1 results in significant increase in cell viability.

On the other hand, overexpression of Tumor Suppressor Gene 1 using *EndoGenius* Inducer Assay (Figure 3A), with no significant change in the expression of another tumor suppressor gene from the same gene family (Figure 3B), results in reduced cell viability (Figure 3C).

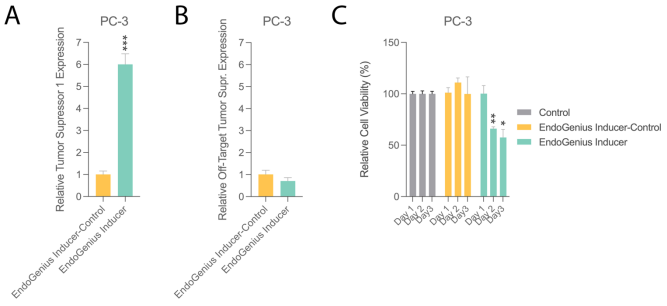

Figure 3. A. *EndoGenius* Inducer specifically induce overexpression of Tumor Suppressor Gene 1, B. with no alteration in another tumor suppressor gene from the same gene family. C. Overexpression of Tumor Suppressor 1 results in significant decrease in cell viability.

It is also possible with *EndoGenius* Inducer to target different genes of a gene family simultaneously. Therefore, the expression of multiple genes can also be easily altered using a single tool.

For further information;
ecotechbiotech.com
info@ecotechbiotech.com

III. Assay Procedure

1. Seed cells so they will be at 40–50% confluency when the *EndoGenius* Inducer Assay is applied to the cells.

2. When the cells are ready, thaw Active Mix and Control Mix on ice.

3. Prepare the following mixtures. Each reaction mix volume is for one well and accounts for pipetting variations. Scale volumes proportionally for additional wells.

	Mix 1	Mix 2	Mix 3	Mix 4
Active Mix	-	-	1 µl	-
Control Mix	1 µl	-	-	-
2x Reaction Buffer	1 µl	-	1 µl	-
Dilution Buffer	150 µl	150 µl	150 µl	150 µl
Encapsulation Buffer	-	1 µl	-	1 µl

4. Combine Mix 1 and Mix 2 in one microcentrifuge tube and label as *EndoGenius* Inducer-Control.

5. Combine Mix 3 and Mix 4 in one microcentrifuge tube and label as *EndoGenius* Inducer.

6. Incubate *EndoGenius* Inducer-Control and *EndoGenius* Inducer mixes from step 4 and 5 at room temperature for 15 minutes.

7. Apply the *EndoGenius* Inducer-Control and *EndoGenius* Inducer to cells in the following volumes.

	96 Well	24 Well	6 Well
EndoGenius Inducer-Control	10 µl	50 µl	300 µl
EndoGenius Inducer	10 µl	50 µl	300 µl

8. Incubate cells for at least 24 hours.

9. Use cells for further assays.

IV. Related Products

EndoGenius Suppressor Assay

*EG*S50 50 Assays
*EG*S100 100 Assays

References

1. Mironov AA, Fickett JW, Gelfand MS. Frequent alternative splicing of human genes. Genome Res **1999**;9:1288-93
2. Rebar EJ, Huang Y, Hickey R, Nath AK, Meoli D, Nath S, *et al.* Induction of angiogenesis in a mouse model using engineered transcription factors. Nat Med **2002**;8:1427-32

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