EndoGenius Suppressor Assay

50 rxn, 100 rxn

Cat No: *EG*S50, *EG*S100

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

I. Assay Components

Components	EGS50 50 Assays	EGS100 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

Drug discovery and development of therapeutic approaches relies heavily on the association of genotypes with phenotypes. One of the best ways to carry out this strategy is to disrupt gene function and then analyze changes in the phenotype. Using RNAi and CRISPR biological tools, researchers can study gene function by suppressing gene expression at the translational or genetic level, respectively (1). However, both systems have certain limitations.

Mammalian systems have evolved a potent antiviral immune response to long double-stranded RNA. This includes the stimulation of interferons and inflammatory cytokines that dramatically alter gene expression and affect a variety of important cellular processes. In particular, siRNAs longer than 23 base pairs trigger strong immune responses that lead to off-target effects and affect functional outputs (2). Certain siRNA sequence motifs, structures, delivery vehicles, and impurities in siRNA preparations can also stimulate immune responses (3). Since siRNA-mediated effects rely on endogenous RNAi mechanisms, overloading the cell with siRNAs will occupy RNAi effector proteins that microRNAs need for gene expression regulation. One study reported that siRNA treatments can lead to significant off-target effects in cells, reporting upregulation of endogenous microRNA targets in a dose-dependent manner corresponding to the amount of siRNA used (4). In a genome-scale RNAi screening study, it was revealed that different siRNAs targeting the same gene produced different phenotypes in cells (5).

Directly regulating the expression of endogenous genes by targeting DNA offers several advantages compared with oligodeoxynucleotides (ODNs) or RNA interference (RNAi) approaches to down-regulate gene expression (6). For downregulation of endogenous genes directly at the DNA level, efficiency is likely to increase as only two copies of DNA per cell need to be targeted compared to the thousands of mRNAs that are usually required to be targeted in RNAi approaches. CRISPRi system also necessitates utilization of large plasmids, technical experience and long optimization processes.

Suppression of endogenous gene expression using specific *EndoGenius* Suppressor Assay results in effective inhibition of all splice variants that is expressed in that specific cell or tissue.

Utilization of *EndoGenius* Suppressor Assay allows inhibition of specific gene expression (Figure 1A) with minimal off-target effects (Figure 1B). It is quite easy to carry out an assay to see functional effects of suppressing an endogenous gene. For example, suppression of a specific oncogene results in significant decrease in viability (Figure 1C).

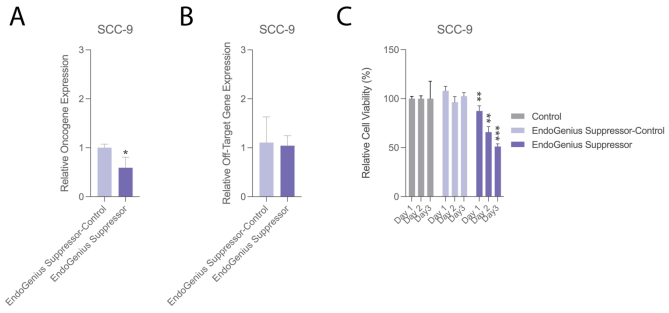

Figure 1. A. *EndoGenius* Suppressor specifically induce significant suppression of Oncogene 1, B. with no alteration in other genes. C. Suppression of Oncogene 1 results in significant decrease in cell viability.

On the other hand, suppression of Tumor Suppressor Gene 1 using *EndoGenius* Suppressor Assay (Figure 2A), with no significant change in the expression of another tumor suppressor gene (Figure 2B), results in increased cell viability (Figure 2C).

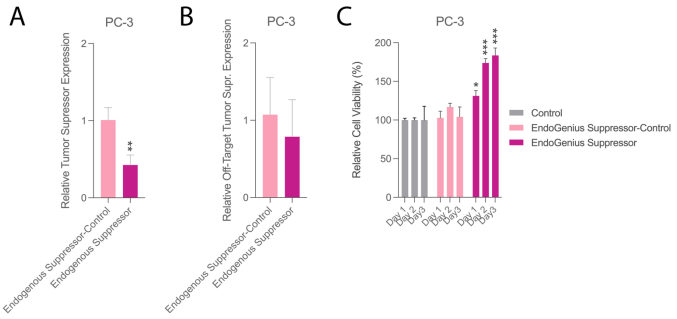

Figure 2. A. *EndoGenius* Suppressor specifically suppressed the Tumor Suppressor Gene 1, B. with no alteration in another tumor suppressor. C. Suppression of Tumor Suppressor 1 results in significant increase in cell viability.

It is also possible with *EndoGenius* Suppressor 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* Suppressor 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* Suppressor-Control.

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

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

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

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

*EGS*50 50 Assays
*EGS*100 100 Assays

References

1. Unniyampurath U, Pilankatta R, Krishnan MN. RNA Interference in the Age of CRISPR: Will CRISPR Interfere with RNAi? Int J Mol Sci **2016**;17:291
2. Reynolds A, Anderson EM, Vermeulen A, Fedorov Y, Robinson K, Leake D, *et al.* Induction of the interferon response by siRNA is cell type- and duplex length-dependent. RNA **2006**;12:988-93
3. https://www.sitoolsbiotech.com/pdf/siRNAofftargeteffects1-170720(1).pdf. siTools.
4. Khan AA, Betel D, Miller ML, Sander C, Leslie CS, Marks DS. Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs. Nat Biotechnol **2009**;27:549-55
5. Marine S, Bahl A, Ferrer M, Buehler E. Common seed analysis to identify off-target effects in siRNA screens. J Biomol Screen **2012**;17:370-8
6. Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, *et al.* Expression profiling reveals off-target gene regulation by RNAi. Nat Biotechnol **2003**;21:635-7

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