

EndoGenius Suppressor Kit

Troubleshooting Guide

50 rxn, 100 rxn

Cat No: EGS50, EGS100

Use this guide for setup and reproducibility problems in the EndoGenius Suppressor Kit workflow. The datasheet specifies separate storage conditions for kit components, 40-50% cell confluency at application, defined control/active mixtures, 15-minute room-temperature complex formation, and at least 24 hours of cell incubation.

Before You Start - Quick Checks

1. Store Active Mix and Control Mix at -20°C, aliquot on receipt, and avoid repeated freeze-thaw; store the other listed buffers at 4-8°C.
2. Seed cells so they are at 40-50% confluency when the kit is applied.
3. Thaw Active Mix and Control Mix on ice.
4. Prepare Mix 1-4 exactly as specified and keep the Control Mix and Active Mix workflows clearly separated.
5. After combining the paired mixtures, incubate at room temperature for 15 minutes.
6. Apply 10 uL per 96-well, 50 uL per 24-well, or 300 uL per 6-well and incubate cells for at least 24 hours.

Problem	Most likely cause	Recommended action
Expected gene suppression is weak	Cells may not have been at the specified 40-50% confluency when the kit was applied.	Repeat the experiment with cells at 40-50% confluency at the time of application.
Active and control conditions look similar	The Active Mix and Control Mix pathways may have been mislabeled or combined incorrectly.	Prepare Mix 1 + Mix 2 as the control condition and Mix 3 + Mix 4 as the active condition, using clearly labeled tubes.
Results vary strongly between experiments	Cell confluency, component handling, or applied volume may not be standardized.	Keep confluency, component storage, 15-minute complex formation, plate format, and applied volume consistent between experiments.
Performance declines after repeated use	Active Mix or Control Mix may have undergone repeated freeze-thaw.	Aliquot Active Mix and Control Mix in small volumes, keep them at -20°C, and avoid repeated freeze-thaw cycles.
Reaction mixture was prepared at the wrong scale	Volumes may not have been scaled proportionally for additional wells.	Use the per-well mixture in the datasheet and scale every component proportionally for the number of wells.
Complex formation may be incomplete	The combined mixtures may not have been incubated for the specified time.	Incubate the combined control and active mixtures at room temperature for 15 minutes before applying them to cells.
Too little or too much mixture is applied	The application volume may not match the culture format.	Use the datasheet application volumes: 10 uL for 96-well, 50 uL for 24-well, and 300 uL for 6-well formats.

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Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Cells are used for readout too early	The post-application incubation may be shorter than specified.	Incubate cells for at least 24 hours before proceeding to the intended functional test.
Unexpected effect is seen in the control condition	The control and active mixtures may have been switched or cross-contaminated.	Reprepare the four mixes in separate labeled tubes and keep Control Mix and Active Mix handling physically distinct.
Buffer storage history is uncertain	The non-active buffers may not have been maintained at the stated 4-8°C storage condition.	Confirm that 2x Reaction Buffer, Dilution Buffer, and Encapsulation Buffer were stored at 4-8°C as specified.
Suppression differs across plate formats	The applied kit volume may not have been adjusted for the plate format.	Use the format-specific application volumes stated in the datasheet and keep cell confluency comparable.
Functional readout is difficult to interpret	The workflow may not have been standardized before the downstream test.	First confirm that the control/active setup, 15-minute complex formation, plate-specific application volume, and at least 24-hour incubation were followed consistently.

Still having trouble? Recheck component-specific storage, 40-50% confluency, the exact control versus active Mix 1-4 setup, 15-minute room-temperature complex formation, plate-format application volume, and the minimum 24-hour incubation before interpreting suppression results.