

Special Note • GeneCopoeia miTarget™ miRNA Target Validation Expression Clones

Dear Researcher,

Thank you for your purchase of GeneCopoeia 3' UTR miRNA target clone(s).

GeneCopoeia 3' UTR miRNA target clones in the pEZX-MT01 vector are not ideally designed for use with the Promega Dual-Glo® Luciferase Assay System.

The GeneCopoeia Luc-Pair™ miR Luciferase Assay Kit has been developed and optimized for use with GeneCopoeia 3' UTR miRNA target clones in the pEZX-MT01 vector.

GeneCopoeia scientists have leveraged the differences in firefly and *Renilla* enzyme structures and substrates resulting in a convenient system for measuring two luciferase activities in succession. The assay measures the activities of firefly and *Renilla* luciferase sequentially from a single sample. **Users should enjoy the following features and benefits included in the Luc-Pair kit design which enhance product performance and add convenience:**

- The system is designed for use on different mammalian cells and is optimized for use on micro-plate readers for high-throughput screening assays as well as for single-tube use.
- The system produces very limited background luminescence (Figure 1). No subtraction is required from readings.
- The Working Solution I (Solution I combined with Substrate I) has all the ingredients necessary for lysing cells in addition to the substrates and stabilizers for the firefly luciferase reaction in a single solution.
- *Renilla* luciferase buffer contains the ingredients to quench firefly luciferase activity from the first step.
- The reagents have been developed so that the signals for firefly and *Renilla* luciferases are relatively stable. Thirty minutes after addition of the appropriate reagent, firefly activity will be at least 80% of its initial activity at 22°C. *Renilla* luciferase activity will be at least 80% of its initial activity after 15 minutes.
- This system is designed to yield reliable, linear results for a concentration range over several orders of magnitude.

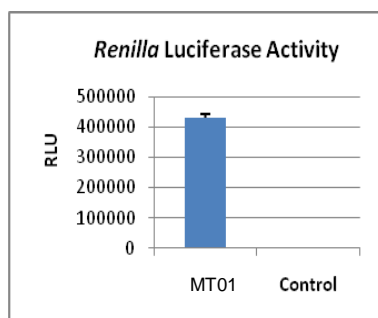
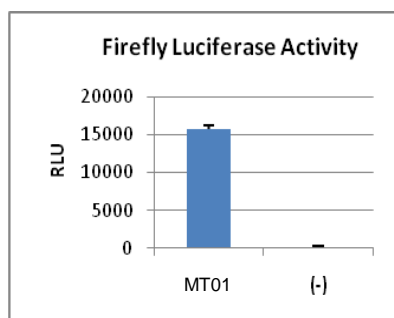


Figure 1. The background of firefly luciferase is low with GeneCopoeia Luc-Pair miR Luciferase Assay. HEK293 cells were plated on a 6-well plate. On the second day, the cells were transfected with either GeneCopoeia pEZX-MT01 target reporter vector or a control plasmid. The cells were transferred to a 96-well plate 18 hours after transfection and cultured for another 24 hours. Then both luciferase assays were performed as described in the procedure. Firefly luciferase activity was measured first (Fig. 1a), followed by *Renilla* luciferase activity (Fig. 1b). Similar results have been obtained on CHO-K1 cells and HeLa cells (Data not shown).

Learn more the benefits and ordering at <http://genecopoeia.com/product/mirna/luciferase-assay-kits.php> or contact support@genecopoeia.com for further information.

Users of the Dual-Glo® luciferase assay should make separate aliquots of the cell lysate and measure the firefly and Renilla luciferase activities independently. Due to the high activity level of Renilla luciferase observed when expressing the miTarget miRNA clones, make a serial dilution of the cell lysate to detect the luminescence at a low enough level when measuring Renilla luciferase activity.