

LightSwitch™ GAS5 Promoter Reporter Vector

Catalog No.: 32072

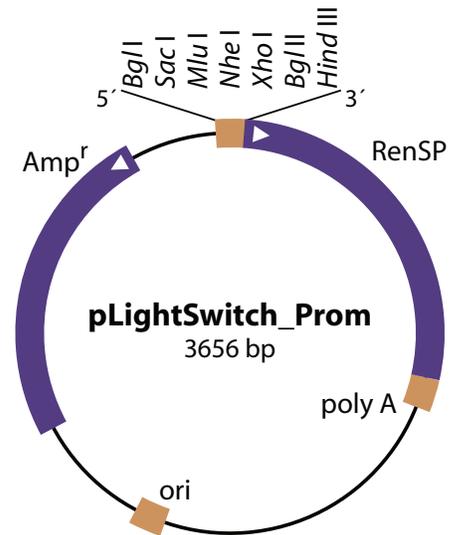
Format: 5 µg

Concentration: ~30 ng/µl

Quality: OD 260/280 ratio ≥ 1.75

Clone & Vector Information: The LightSwitch GAS5 Promoter Reporter Vector contains 1078 bp of GAS5 lncRNA promoter sequence cloned from the human genome into the pLightSwitch_Prom reporter vector (right) using the *Mlu* I and *Bgl* II sites, upstream of the RenSP luciferase gene. LightSwitch vector maps, annotations, and sequence & primer information are available at www.activemotif.com/ls-vectors. Sequence information for the GAS5 lncRNA promoter can be found by clicking the “Get Info” link for this construct in the ordering table found at www.activemotif.com/ls-lncrna.

LightSwitch Assays: Because all LightSwitch Reporter constructs utilize the RenSP luciferase reporter gene, you **MUST** use the LightSwitch Luciferase Assay Kit (Cat. Nos. 32031 & 32032) to perform luciferase assays with all LightSwitch vectors. This kit contains a proprietary substrate that was formulated specifically for use with our engineered RenSP gene. Other luciferase assay reagents are **NOT** compatible with RenSP. For more information on the LightSwitch Assay Kit, please go to www.activemotif.com/ls-assay.



Use of a Positive Control for Normalization: When performing LightSwitch assays of lncRNA promoters, it is important to normalize your results to a positive control sample, such as the ACTB Promoter Control (Cat. No. 32003), if you are performing any type of treatment on your cells. Many of the treatments used to induce lncRNA promoters can reduce cell proliferation or cause growth arrest or apoptosis/necrosis to occur. In all of these cases, luciferase expression from a control promoter will also be decreased compared to control promoter samples that are not being treated. By normalizing the lncRNA promoter samples to control promoter samples assayed under the same treatment conditions you can negate the effects that cell growth arrest and cell death have on your results. Perform your assays in triplicate and divide the average values of the lncRNA promoter (both treated and untreated) by the average values of the control promoter, then multiply by 100. The empty pLightSwitch_Prom reporter vector can be used to measure background signal.

Transfection Reagents: We recommend FuGENE® HD Transfection Reagent (Cat. Nos. 32042 & 32043) for all plasmid transfections as it has superior efficiency and low cytotoxicity across a wide variety of cell lines. If you are co-transfecting a plasmid together with a short RNA (siRNA or miRNA), we recommend DharmaFECT® Duo (Cat. Nos. 32044 & 32045).

If you have not yet optimized the transfection conditions for your cell line or treatment condition of interest, we offer the LightSwitch Transfection Optimization Kit (Cat. No. 32041), which includes FuGENE, LightSwitch Positive & Negative Control Reporter constructs and a LightSwitch Luciferase Assay Kit. Use of this kit will help you consistently achieve higher transfection efficiencies, which will greatly improve the results of your luciferase reporter assay experiments. For more information, please go to www.activemotif.com/transfect.

Single vs. Dual Assay Design: Modern transfection reagents and optimized luciferase assay reagents such as LightSwitch have largely eliminated the need to normalize results through use of co-transfection controls. In most cases, using a dual assay format provides little benefit, while increasing costs and reducing assay sensitivity. Unless you are using a hard-to-transfect cell line, we recommend that you first try using a single transfection format to determine if there is extensive variation between transfection replicates of your cell line. If there is, you can normalize by co-transfection using the LightSwitch Dual Assay Kit (Cat. No. 32035), which has been optimized for use with all LightSwitch reporter vectors. For more information on the pros and cons of co-transfection, please download a PDF of our Technical Note on the subject by entering www.activemotif.com/ls-co-trans into your browser.