

LightSwitch 5'UTR Reporter Vector

Catalog No: 32023 – **Product ID:** S690005

Concentration: ~30 ng/μl

Quality: OD 260/280 ratio ≥ 1.75

Vector Information: The LightSwitch 5'UTR Reporter Vector can be used to test how various 5'UTR sequences effect transcription from a downstream promoter. DNA fragments that contain 5'UTR sequences are cloned into the Multiple Cloning Site (MCS) of the pLightSwitch_5UTR vector upstream of an ACTB promoter & 5'UTR, and the RenSP luciferase reporter gene. The cloned fragment will be constitutively expressed as part of a hybrid transcript that contains the 5'UTR sequence of interest fused to the coding sequence of RenSP. LightSwitch vector maps, annotations, and sequence & primer information are available at www.activemotif.com/ls-vectors.

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, please go to www.activemotif.com/ls-assay.

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

Positive & Negative Controls: We recommend that you include appropriate positive & negative control LightSwitch vectors when you perform your assays. We offer a panel of human housekeeping gene promoters as positive controls. In most cases, the ACTB Promoter Control (Cat. No. 32003) is a suitable positive control. Other housekeeping gene promoters are also available. The empty pLightSwitch_Prom reporter vector can be used to measure background signal.

Single vs. Dual Assay Design: Modern transfection reagents and optimized luciferase assay reagents such as LightSwitch have largely eliminated the need to do a co-transfection control. 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 testing the variation between transfection replicates in a single transfection format. If you wish to do a co-transfection, we offer 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 our Technical Note by entering www.activemotif.com/ls-co-trans into your browser.

