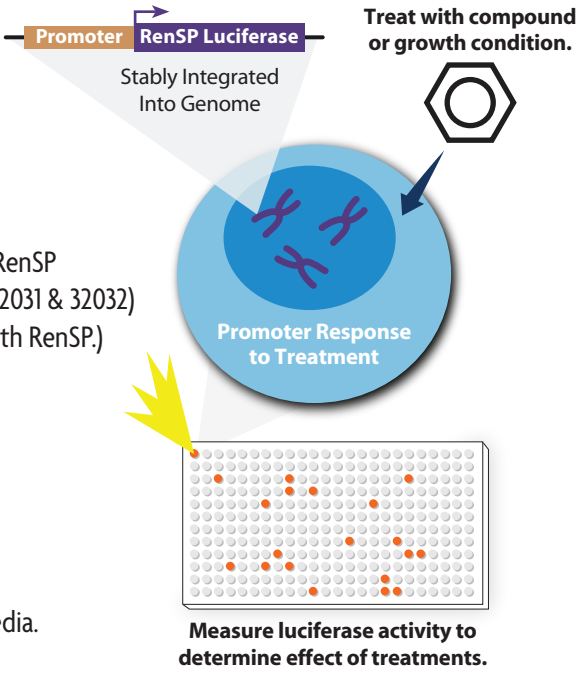
NFκB v3 Reporter Cell Line (HT1080)

Catalog No.: 32216

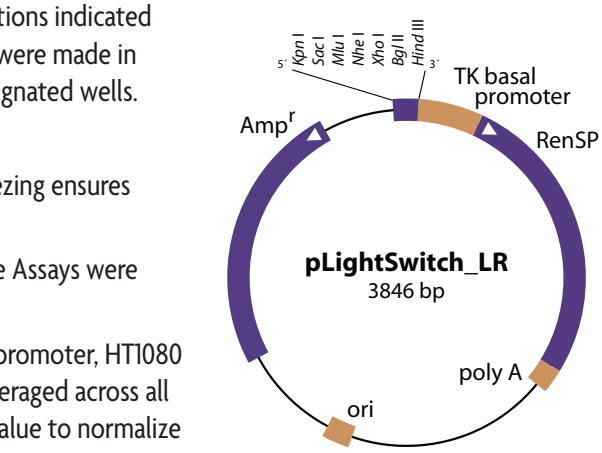
The LightSwitch™ NFκB v3 Reporter Cell Line (HT1080) was designed for study of the Inflammation pathway. It contains a stably integrated LightSwitch Synthetic Response Element reporter construct (S900018), which is comprised of repeats of an NFκB binding site motif, cloned upstream of a minimal promoter and the RenSP luciferase gene in the Long-range Enhancer Reporter Vector, **pLightSwitch_LR vector**.

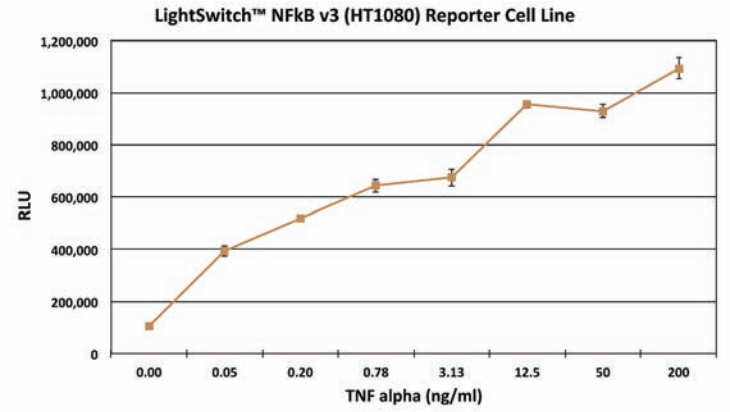


IMPORTANT: Because all LightSwitch reporter cell lines contain the optimized RenSP luciferase gene, you MUST use our **LightSwitch Luciferase Assay Kit** (Cat. Nos. 32031 & 32032) to obtain optimal results. (Other luciferase assay reagents are not compatible with RenSP.)

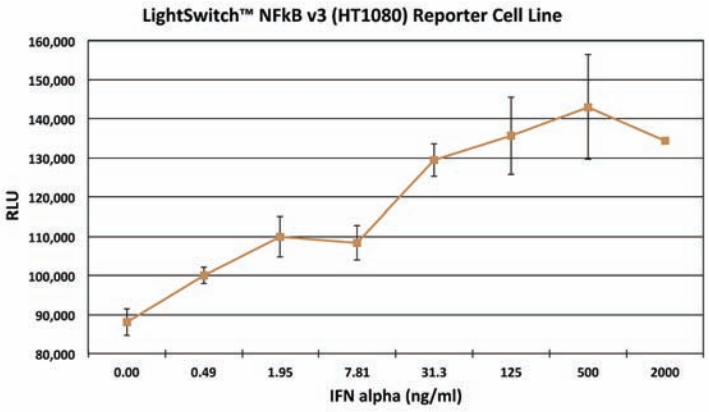
Experimental Details:

1. Assays were performed in triplicate. 10K cells per well were seeded in a 96-well white plate in standard media without antibiotic.
2. Cells were incubated at 37°C for 8 hours.
3. Standard media was removed and replaced with 100 µl of OptiMEM media. The cells were then incubated at 37°C overnight.
4. 24 hours post-seeding, cells were induced with the treatment conditions indicated below. Two 10X dose response series of TNF alpha and of IFN alpha were made in OptiMEM. Ten µl of the indicated 10X stocks were added to the designated wells.
5. Cells were incubated at 37°C for 8 hours.
6. Plates were frozen at -80°C overnight. (This step is optional, but freezing ensures complete lysis of the cells prior to running the LightSwitch Assay.)
7. Plates were thawed to room temperature and LightSwitch Luciferase Assays were performed per the standard protocol.
8. The data was normalized to the Control 2 Reporter Cell Line (ACTB promoter, HT1080 cells; Cat. No. 32202); expression data for the control cell line was averaged across all doses. Experimental data points were then divided by this average value to normalize for non-specific effects.





T-Test: 1.14E-07
Z´: 0.94



T-Test: 4.7E-05
Z´: 0.75