## NFKB v1 Reporter Cell Line (HT1080)



Treat with compound

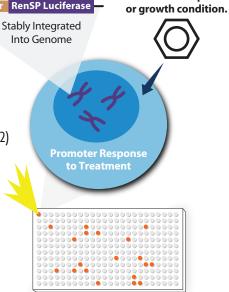
**Catalog No.: 32214** 

The LightSwitch™ NFkB v1 Reporter Cell Line (HT1080) was designed for study of the Inflammation pathway. It contains a stably integrated LightSwitch Promoter Reporter GoClone™ construct (S721709), which is comprised of the promoter sequence of the IL8 (aka CXCL8, chemokine (C-X-C motif) ligand 8) gene cloned into the MCS of the Promoter Reporter Vector, pLightSwitch\_Prom, upstream of the RenSP luciferase gene.

**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:**

- 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  $\mu$ l of OptiMEM media. The cells were then incubated at 37°C overnight.
- 24 hours post-seeding, cells were induced with the treatment conditions indicated below. A 10X dose response series of TNF alpha was made in OptiMEM. Ten µl of the indicated 10X stock was added to 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.



Measure luciferase activity to determine effect of treatments.

