

SuperLight™ Luciferase Reporter Gene Assay Kits

Bioluminescent Assay for Promoter Regulated Luciferase Expression

of cells per well be determined by serial dilution of cells. Cells can be adherent or in suspension cultures.

Cell lysis and mixing. For the sake of convenience, the addition of 1 volume of reconstituted reagent to 1 volume of cells allows a sufficient mixing. No additional mixing is required since the specially formulated buffer instantly lyses mammalian cells.

DATA ANALYSIS

The light intensity (RLU) is directly proportional to the luciferase concentration. For dose-response studies, the data are plotted against compound concentration and the EC_{50} for gene up-regulator compound and IC_{50} for a gene down regulator compound can be determined by non-linear regression analysis using Prism or other data analysis tools.

LITERATURE

Gene Regulation (receptors, small molecules, transcription factors)

- Imaki H et al (2003). Cell cycle-dependent regulation of the Skp2 promoter by GA-binding protein. *Cancer Res.* 63(15):4607-13.
- Nabors LB et al (2003). Tumor necrosis factor alpha induces angiogenic factor up-regulation in malignant glioma cells: a role for RNA stabilization and HuR. *Cancer Res.* 63(14):4181-7.
- Keiss HP et al (2003). Garlic (*Allium sativum* L.) modulates cytokine expression in lipopolysaccharide-activated human blood thereby inhibiting NF-kappaB activity. *J Nutr.* 133(7):2171-5.

Characterization of promoter activity

- Sharina IG et al (2003). CCAAT-binding factor regulates expression of the beta1 subunit of soluble guanylyl cyclase gene in the BE2 human neuroblastoma cell line. *Proc Natl Acad Sci U S A.* 100(20):11523-11528.
- Cramer SD et al (2003). Association between genetic polymorphisms in the prostate-specific antigen gene promoter and serum prostate-specific antigen levels. *J Natl Cancer Inst.* 95(14):1044-53.

Modulation of gene expression by small molecules

- Kim SH et al (2003). Luteolin inhibits the nuclear factor-kappa B transcriptional activity in Rat-1 fibroblasts. *Biochem Pharmacol.* 66:955-63.

High-throughput screening for gene modulators Reviews:

- Phippard D, Manning AM (2003). Screening for inhibitors of transcription factors using luciferase reporter gene expression in transfected cells. *Methods Mol Biol.* 225:19-23.
- Naylor LH (1999). Reporter gene technology: the future looks bright. *Biochem Pharmacol.* 58:749-57.

cAMP response element (CRE), NFAT and SRE dependent pathways:

- Chen G et al (1999). Constitutive receptor systems for drug discovery. *J Pharmacol Toxicol Methods.* 42:199-206.
- Baldari CT et al (1998). NF-AT-luciferase reporter T cell lines as tools to screen immunosuppressive drugs. *Biologicals.* 26:1-5.
- Choi SE, Choi EY, Kim PH, Kim JH. Involvement of protein kinase C and rho GTPase in the nuclear signalling pathway by transforming growth factor-beta1 in rat-2 fibroblast cells. *Cell Signal.* 1999;11(1):71-6.
- Rapisarda A et al (2002). Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res.* 62:4316-24.

TECHNICAL NOTES

The SuperLight™ Luciferase Reporter Gene Assay Kit has been specially optimized and formulated to provide a sensitive, convenient and robust assay for gene expression and regulation studies in mammalian cells. Key features of the kits are as follows:

High sensitivity and wide detection range: detection of as little of 2 fg luciferase and as few as 4 cells. Plus, the emitted light is linear over seven orders of magnitude.

Compatible with routine laboratory and HTS formats: assays can be performed in tubes or microplates, on LJL Analyst, Berthold Luminometer, Top-Count, MicroBeta counters, chemiluminescent image plate readers (CLIPR/LeadSeeker). Assay reagents compatible with all liquid handling systems.

Fast and convenient: homogeneous "mix-and-measure" assay allows detection of luciferase levels within 10 minutes. The optimally combined reagent system allows a single addition step and simultaneous cell lysis and detection.

Robust and amenable to HTS. Z' factors of 0.7 to 0.9 are routinely observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems.

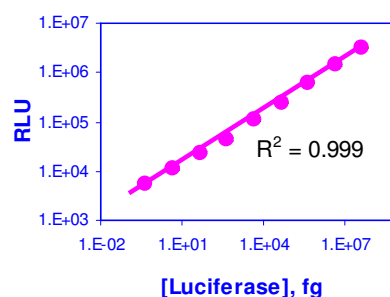


Figure 1. Linearity of the SuperLight™ Luciferase Assay in 384-well plate. The detection limit estimated from the blank controls was 2 fg. Light intensity was linear from 2 fg up to 46 ng.

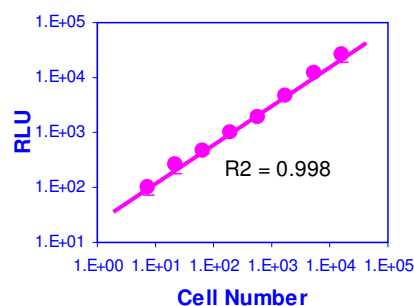


Figure 2. Linear relationship between emitted light and number of HEK293 cells transiently transfected with a CRE-luciferase reporter construct in a 384-well plate assay. Detection limit: 4 cells.

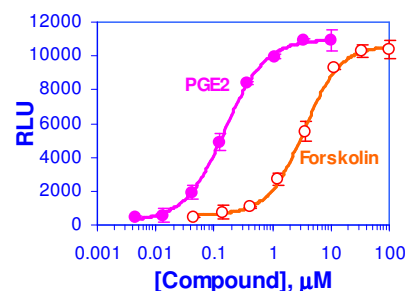


Figure 3. Up-regulation of CRE-dependent luciferase expression by prostaglandin E2 (PGE₂) and adenylyl cyclase activator forskolin in HEK293 cells transiently transfected with a CRE-luciferase construct. EC_{50} = 0.15 μM for PGE₂ and 3.5 μM for forskolin.