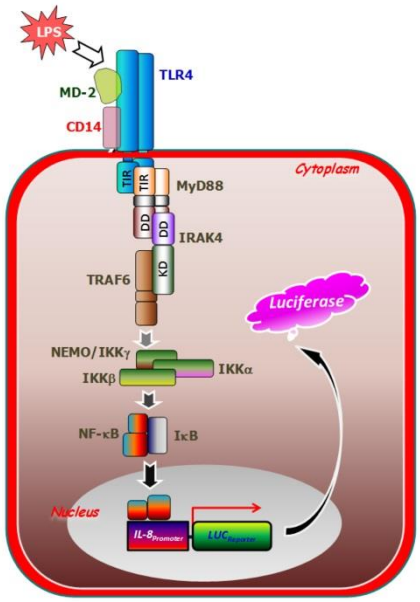
Engineered cell lines which stably express SEAP or Luciferase reporter proteins

Reporter cell lines provide a simple, rapid and reliable method of monitoring the activity of signalling pathways. Cells are engineered to express a reporter gene downstream of the promoter or regulatory sequence of the gene of interest (Figure 1). The encoded reporter protein is then quantified in response to an appropriate stimulus.



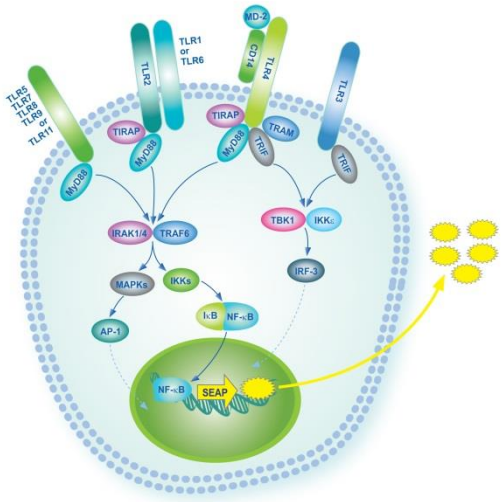


Figure 1a. **Figure 1b.**

Figure 1a. The LUCPorter™ cell lines express the luciferase reporter gene under the transcriptional control of various promoters or response elements. **Figure 1b.** The SEAPorter™ cell lines express the reporter gene for SEAP under the transcriptional control of an NF-κB response element.

A good reporter protein should be easily detectable and not normally be present in the organism being investigated. Well-studied reporter proteins include luciferase, secreted alkaline phosphatase (SEAP), β-galactosidase and Green Fluorescent Protein (GFP). The reporter protein can itself be directly measured; alternatively its enzymatic activity can be quantified. Novus Biologicals specializes in LUCPorter™ and SEAPorter™ reporter cell lines for studying inflammation, autoimmune disease and cancer-related signalling pathways including NF-κB, TLR, AP-1, NFAT, GATA3 and STAT pathways.

LUCPorter™ cell lines

Although firefly luciferase has long been used as a reporter gene, marine luciferases have become popular alternatives. Firefly luciferase uses luciferin in the presence of oxygen, ATP and magnesium to produce green-yellow light in the 550-570nm range. In contrast, the LUCPorter™ cell lines express

the luminescent reporter gene RenSP from *Renilla reniformis,* a sea pansy marine animal which emits blue-green bioluminescence at 480nm. *Renilla* luciferase requires only coelenterazine and oxygen as substrates. The small size of the *Renilla* gene and protein (936bp and 36kDa), along with its lack of dependence on ATP, provide a distinct advantage over the larger (1.6kb and 62kDa) ATP-dependent firefly luciferase. A one-step addition of a luciferase assay reagent enables the measurement of luciferase activity by producing a bright and stable signal that is easily quantifiable, sensitive and amenable to high throughput screening.

Novus Biologicals currently offers more than twenty LUCPorter™ cell lines (Figure 2). These are human or mouse cells that have been stably transfected with a vector encoding the *Renilla* luciferase reporter gene under the transcriptional control of various promoters or response elements, including those of IL17A, RORγ and STAT3. These reporter cell lines can be used to screen for agonism (activation) or antagonism (inhibition of an agonist) of the cell signal mediators or transcription factors that can induce promoter activity. This is readily accomplished by simply treating the cells with test compounds, followed by lysis and the measurement of reporter activity using the LightSwitch Luciferase Reporter Assay Reagent (catalog no. NBP2-25287). This assay system is optimized to pair with all of the LUCPorter™ cell lines.

Human			Mouse	
Target gene	**Promoter**	**Catalog number**	**Promoter**	**Catalog number**
	AP-1 RE	NBP2-26282	CXCL2	NBP2-26250
	CXCL2	NBP2-26249	IL-8	NBP2-26291
	FOXP3	NBP2-26294	NFAT RE	NBP2-29351
	GATA3 RE	NBP2-26251	NF-κB RE	NBP2-26253
	IL-17A	NBP2-26283	TNFα	NBP2-26256
	NFAT RE	NBP2-26252	TNFβ	NBP2-26257
	RORγ	NBP2-26284	STAT1 RE	NBP2-29352
	RORγ T	NBP2-26285		
	STAT1 RE	NBP2-29634*		
	STAT3 RE	NBP2-26254		
TLR3	IFNβ	NBP2-26262		
TLR3	Interferon-sensitive RE	NBP2-26292		
TLR4, MD-2, CD14	IL-8	NBP2-26263*		
	TNFα	NBP2-26255		
	TNFβ	NBP2-26258		
	eIF4E	NBP2-30093		
	HER2	NBP2-30092		

Figure 2. LUCPorter™ cell lines. Human lines are HEK293 unless marked * for HeLa. Mouse lines are RAW264.7. RE = Response element. Note: A human vector control LUCPorter™ cell line (catalog no. NBP2-29350) is also available; this control cell line contains the luciferase reporter gene but lacks a promoter, hence luciferase activity is not inducible.

SEAPorter™ cell lines

SEAPorter™ cells are also stably transfected HEK293 cells, however they rely on secreted alkaline phosphatase (SEAP) rather than luciferase as the reporter gene. SEAP catalyses the hydrolysis of pNitrophenyl phosphate (pNPP), converting it to a coloured product, the absorbance of which can then be read in a spectrophotometer at 405nm. Since SEAP is secreted by cells, lysis is not required for its detection. This enables kinetic experiments to be performed through sequential sampling of the media, and also allows the cells to be used for additional purposes such as Western blotting or RNA extraction.

Novus Biologicals currently offers more than 10 SEAPorter™ cell lines, which have been stably co-transfected with TLR and SEAP genes that are under the transcriptional control of an NF-κB response element (Figure 3). The SEAPorter™ cell lines can be used to screen for TLR agonists and antagonists. SEAP activity is measured with the Secreted Alkaline Phosphatase Reporter Assay Kit (catalog no. NBP2-25285), which is optimized to pair with all of the SEAPorter™ cell lines. Additionally, Novus Biologicals offers a unique NF–κB Secreted Alkaline Phosphatase Assay Kit (NBP2-25286), that includes an NF-κB/SEAP plasmid for researchers who want to construct their own SEAP reporter cell lines.

Target gene	Promoter	Catalog number
	NF-κB RE	NBP2-26260
TLR2	NF-κB RE	NBP2-26274
TLR3	NF-κB RE	NBP2-26275
TLR4	NF-κB RE	NBP2-26276
TLR4, MD2, CD14	NF-κB RE	NBP2-26503
TLR5	NF-κB RE	NBP2-26277
TLR7	NF-κB RE	NBP2-26278
TLR8	NF-κB RE	NBP2-26279
TLR9	NF-κB RE	NBP2-26280
TLR10	NF-κB RE	NBP2-26273
TLR11	NF-κB RE	NBP2-26289
TLR12	NF-κB RE	NBP2-26288
TLR13	NF-κB RE	NBP2-26290

Figure 3. SEAPorter™ cell lines. RE = Response element

Reporter cell line applications - summary

Reporter cell lines are powerful tools for evaluating gene activity, especially when studying complex signalling such as that exhibited by the TLR pathway. The SEAPorter™ cell lines are designed for the analysis of cellular responses that result in modulation of NF-κB activities, and enable the TLR

specificity of agonists, or antagonists, to be investigated. The LUCPorter™ cell lines provide coverage of a broader range of signalling pathways.

Homogeneous bioluminescent assays can rapidly be developed using reporter cell lines, and are easily amenable to multiwell plate formats and automation. Reporter cell line assays also provide a simple and sensitive screening platform for the evaluation of agonistic or antagonistic effects and, in the case of secreted reporter proteins such as SEAP, are an extremely powerful tool for kinetic analysis. Unlike conventional antibody-based analyses, reporter gene assays are quantifiable, have a large dynamic range, and are amenable to kinetic analysis.

TLR signalling

Toll-like receptors (TLRs) play a pivotal role in initiating innate immune responses following exposure to conserved microbial components such as lipopolysaccharide and dsRNA. Thirteen mammalian TLRs are currently known; these bind and become activated by various different ligands, resulting in signalling cascades that ultimately trigger activation of transcription factors and expression of genes involved in the immune response (1).

NF-κB is involved in controlling the immune response to infection, and incorrect regulation of this protein is implicated in cancer, inflammatory disorders and autoimmune conditions. In resting cells, NF-κB is sequestered by inhibitor of κB (I-κB). Activation of TLR signalling pathways results in phosphorylation of I-κB, leading to its ubiquitination and degradation, allowing the released NF-κB to translocate to the nucleus, where it binds to NF-κB response elements and recruits other proteins to activate the transcription of target genes (2).

1) PMID: 24083080
2) PMID: 23495970