

InvivoGen Insight

SUMMER
2012

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Introducing Lucia™: a new secreted luciferase



Discover InvivoGen's Brightest Tool

This issue puts the spotlight on InvivoGen's latest development, Lucia™, a new secreted luciferase optimized to advance cell based reporter technology.

Luciferases encompass a wide range of enzymes used for bioluminescence, the emission of light produced by a living organism. Luciferases are highly prized bioindicators for life science research and drug discovery, owing to their remarkable sensitivity, lack of toxicity and wide dynamic range of quantitation. Luciferases are used for many bioluminescence applications including gene reporter assays, whole-cell biosensor measurements, protein interaction studies using bioluminescence resonance energy transfer (BRET), drug discovery through high throughput screening and *in vivo* imaging.

The best studied luciferases, derived from the firefly and the sea pansy *Renilla*, are intracellular reporters and are associated with the need to lyse cells in order to measure bioluminescence. One of the several advantages of secreted luciferases is the ease of detection directly from the cell culture medium, enabling kinetic studies from the same cells. Despite their many advantages, secreted luciferases are overshadowed by the traditional firefly and *Renilla* luciferases.

Secreted Luciferases

Naturally secreted forms of luciferases from marine bioluminescent organisms have been known for over 100 years, although their identification is relatively recent. The first secreted luciferase to be cloned was isolated from the marine ostracod crustacean, *Vargula hilgendorffii*, formerly known as *Cypridina hilgendorffii*, of the Cypridinidae family¹. Later, secreted luciferases were isolated from luminous glands of marine copepod crustaceans *Gaussia princeps*², *Metridia longa*³ and *Metridia pacifica*⁴ of the Metridinidae family. Earlier this year, newly identified secreted luciferases

were cloned from copepods of Heterorhabdidae, Lucicutiidae and Augaptilidae families, isolated from Zooplankton collected off the deep sea of Japan⁵. The secreted copepod luciferases were functionally tested, and sequence alignment of the genes revealed a high degree of similarity in the primary structure. Copepod luciferases share two catalytic domains, D1 and D2, and an amino-terminal signal sequence.

Conveniently, when expressed in mammalian or insect cells, the native signal sequences of these luciferases are functionally active, mediating their export from within the cell to the surrounding culture medium. Bioluminescence assays are simply conducted using culture media, whereupon the activity of the secreted luciferases provides a readout of the biological signaling event under study. This aspect together with the strong intensity of bioluminescent signal generated, make secreted luciferases appealing for the design of novel reporter genes with enhanced properties⁶⁻⁸. Applications of secreted luciferases extend beyond their use as *in vitro* cell biological reporters, and can be adapted for real-time *ex vivo* monitoring of *in vivo* biological processes⁹⁻¹¹.

InvivoGen's Lucia™ is a completely novel secreted luciferase expressed by a synthetic gene designed on the naturally secreted luciferases from marine copepods. Lucia™ has been engineered for its superior properties compared to natural secreted luciferases. The superior bioluminescence signal generated by Lucia™ luciferase is magnitudes stronger than the commonly used firefly and *Renilla* luciferases. The intense bioluminescence facilitates real-time measurements to detect very small amounts of the reporter in the cell culture medium and slight changes in the reporter concentration. Furthermore, the Lucia™ gene is codon

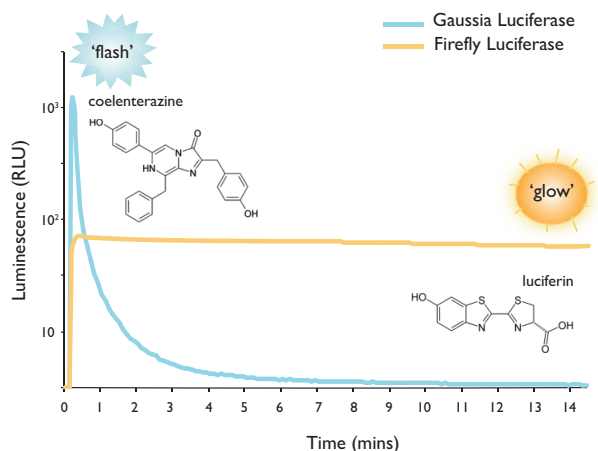
Luciferase (Species)	Secreted	Size kDa	Emission type (T _{1/2})
Luciferin utilizing luciferases			
<i>Photinus pyralis</i> (firefly)	no	61	Glow
<i>Vargula hilgendorffii</i>	yes	62	Glow
Coelenterazine utilizing luciferases			
Lucia™	yes	23	Extended flash (5 mins)
<i>Renilla reniformis</i>	no	36	Flash (30 sec)
<i>Gaussia princeps</i>	yes	20	Flash
<i>Metridia longa</i>	yes	24	Flash
<i>Metridia pacifica</i>	yes	20-23	Flash



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optimized and free of CpG dinucleotides for prolonged mammalian cell expression. A major challenge in the use of cell-based assays is establishing cell lines that reliably express a reporter gene without experiencing diminishing expression with increasing passages. Expression of the Lucia™ gene is designed for stable expression providing reliability and biological significance between experiments.



Bioluminescence signals produced by coelenterazine- and luciferin-utilizing luciferases

Luciferase Detection Reagents

The phenomenon of bioluminescence is the emission of visible light produced when luciferases catalyze the oxidation of specific substrates. Substrates of luciferases can be broadly classed into two groups; luciferins and coelenterazines. Luciferases, such as firefly, that use luciferin or derivatives as substrates require ATP and Mg^{2+} as cofactors and display stable "glow" kinetics. The light emitted is generally in the green-yellow region of the visible light spectrum. Conversely, luciferases using coelenterazine do not require ATP for activity and produce a rapid, often intense, "flash" light. *Renilla* luciferase along with the secreted copepod luciferases known to date display substrate specificity toward coelenterazine. The coelenterazine-utilizing luciferases emit visible blue light with a wavelength between 465-493 nm.

Coelenterazine, as a molecule, is unstable with respect to its auto-oxidation potential. Spontaneously, coelenterazine is oxidized to coelenteramide yielding the visible blue light and release of carbon dioxide. A number of variants and synthetic analogs have been used to improve stability by preventing auto-oxidation, in order to reduce background signal during assaying^{1,2}.

InvivoGen's QUANTI-Luc™ is a one-step detection reagent paired for use with Lucia™. QUANTI-Luc™ contains coelenterazine with stabilizing agents that limits substrate auto-oxidation and allows for the reconstituted reagent to be stored. When used in combination with Lucia™, the bioluminescent flash signal generated is longer-lasting, providing flexibility for taking readings. An expanding number of products are offered by InvivoGen creating a Lucia™ product portfolio (see below). InvivoGen offers reporter cell lines expressing the Lucia™ reporter gene, either alone or in combination with a secreted embryonic alkaline phosphatase (SEAP) reporter gene, allowing for concomitant monitoring of two distinct signaling pathways. Additionally, InvivoGen offers Lucia™ in a variety of mammalian expression plasmids, the recombinant protein and an anti-Lucia™ antibody. InvivoGen's Lucia™ product line is designed to introduce ease and stretch the sensitivity in your reporter systems. Let the light of Lucia™ into your experiments!

1. Thompson EM, et al., 1989. Cloning and expression of cDNA for the luciferase from the marine ostracod *Vargula hilgendorfii*. Proc Natl Acad Sci U S A 86: 6567-71. 2. Verhaegent M. & Christopoulos TK., 2002. Recombinant *Gaussia* luciferase. Overexpression, purification, and analytical application of a bioluminescent reporter for DNA hybridization. Anal Chem 74: 4378-85. 3. Markova SV, et al., 2004. Cloning and expression of cDNA for a luciferase from the marine copepod *Metridia longa*. A novel secreted bioluminescent reporter enzyme. J Biol Chem 279: 3212-17. 4. Takenaka Y, et al., 2008. Two forms of secreted and thermostable luciferases from the marine copepod crustacean, *Metridia pacifica*. Gene 425: 28-35. 5. Takenaka Y, et al., 2012. Evolution of bioluminescence in marine planktonic copepods. Mol Biol Evol 29(6):1669-81. 6. Kim SB, et al., 2011. Superluminescent Variants of Marine Luciferases for Bioassays. Anal Chem. 83(22):8732-40. 7. Maguire CA, et al., 2009. *Gaussia* luciferase variant for high-throughput functional screening applications. Anal Chem 81: 7102-06. 8. Enjalbert B, et al., 2009. A multifunctional, synthetic *Gaussia* princeps luciferase reporter for live imaging of *Candida albicans* infections. Infect Immun 77: 4847-58. 9. Wurdinger T, et al., 2009. A secreted luciferase for ex vivo monitoring of in vivo processes. Nat Methods, 5(2): 171-173. 10. Tannous BA. & Teng J., 2011. Secreted blood reporters: Insights and applications. Biotechnol Adv 29: 997-1003. 11. Ray P. & Gambhir SS., 2007. Noninvasive imaging of molecular events with bioluminescent reporter genes in living subjects. Methods Mol Biol 411: 131-144. 12. Zhao H., 2004. Characterization of coelenterazine analogs for measurements of *Renilla* luciferase activity in live cells and living animals. Mol Imaging 3(1):43-54.

Lucia™ Product Line

Lucia™ Reporter Gene System

- Lucia™ Reporter Gene
- Recombinant Lucia™ Protein
- Anti-Lucia™ -IgG
- QUANTI-Luc™, Lucia™ detection reagent

Lucia™ Reporter Plasmids

- pNiFty-Lucia, PRR signaling reporter plasmids

Lucia™ Reporter Cell Lines

- HEK-Dual™ TNF- α , TNF- α Lucia™/SEAP reporter cells
- HEK-Dual™ IFN- γ , IFN- γ Lucia™/SEAP reporter cells
- THPI-Dual™ (NF- κ B - ISG), NF- κ B-SEAP & IRF-Lucia™ reporter monocytes
- Jurkat-Dual™ (ISG - NF- κ B), NF- κ B-Lucia™ & IRF-SEAP reporter T lymphocytes



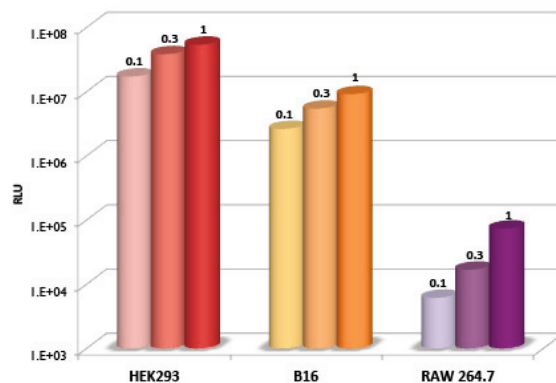
Lucia™ & QUANTI-Luc™

Setting a new standard in reporter gene systems

In vivoGen has developed Lucia™, a new secreted luciferase, in combination with QUANTI-Luc™, an innovative luciferase reporter reagent. Together, they provide a powerful and yet easy-to-use tool for a wide variety of applications, including promoter studies, transfection optimization studies, signal transduction studies, kinetic studies and multiple assays with other reporters.

Properties of Lucia™

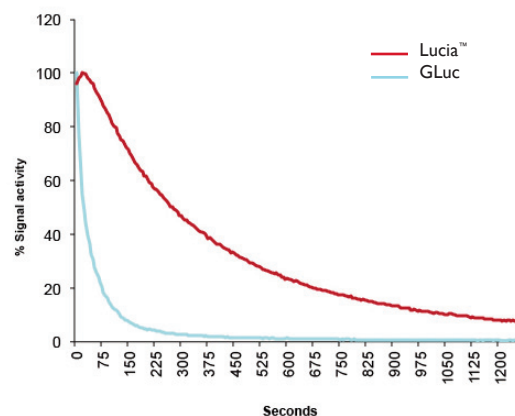
- **Efficiently secreted by mammalian cells**
 - Preparation of cell lysates is not required. Only small aliquots of the culture media are needed to assess Lucia™ activity
 - Kinetics of gene expression can be easily studied by repeated collection of medium from the same cultures
 - Transfected cells are not disturbed during measurements of Lucia™ activity and remain intact for further investigations
- **Extreme sensitivity**
 - 1000X brighter than Firefly and *Renilla* luciferases
 - Results are obtained earlier than conventional reporters
 - Low levels of expression can be detected
 - Amenable to difficult to transfect cell lines
- **No endogenous activity**
 - Low background enhancing assay sensitivity
- **Persistent gene expression**
 - Lucia is encoded by a CpG-free gene limiting gene silencing and maintaining expression with repeated cell passages
- **Enhanced stability**
 - Cell supernatants can be stored for several days at 4°C or several weeks at -20°C with no loss of Lucia™ activity



High signal sensitivity. The human embryonic kidney HEK293, the murine melanoma B16 and the murine macrophage RAW 264.7 cell lines were transiently transfected with 0.1, 0.3 or 1 µg/ml of pSELECT-Lucia-zeo plasmid using the transfection reagent LyoVec™. Forty-eight hours after transfection, cell supernatants were assessed for Lucia™ activity using QUANTI-Luc™.

Features of the Lucia™/QUANTI-Luc™ Assay

- **One step reagent**
 - Contains all the components required for the quantification of Lucia™ reporter activity
 - Just add water - No additional reagents required
- **Highly convenient & cost effective**
 - Provided lyophilized in individually sealed pouches
 - Shipped at room temperature - No need for dry ice
 - Each pouch prepares 5 x 96 well plates
- **Stable reconstituted reagent**
 - Reconstituted QUANTI-Luc™ can be stored 8 hrs at room temperature (RT), 1 week at 4°C or 1 month at -20°C without significant loss of activity
- **Coelenterazine-utilizing assay**
 - Provides maximum brightness
 - ATP independent reaction
- **Low autoluminescence background**
- **Extended-flash kinetics**
 - 10X more stable than *Gussia* luciferase (GLuc) signal
 - Half-life ~5 mins at RT, 10% signal decay in 75 secs
 - Allows signal measurement in luminometers without injectors when using 96-well plates
- **Endpoint measurement**
 - Lucia™ enhanced signal stability allows for endpoint reading as an alternative to kinetic reading. - Shortens time-to-results



Signal decay kinetics. HEK293 cells were transiently transfected with a pSELECT-zeo plasmid expressing Lucia or GLuc. Forty-eight hours after transfection, cell supernatants were transferred to a white 96-well plate and assessed for luciferase activity using QUANTI-Luc™.

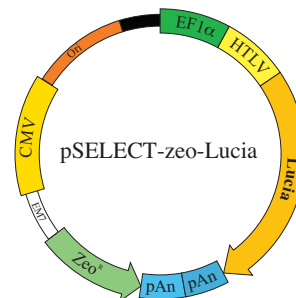
Lucia™ Reporter Gene System

Lucia™ Reporter Gene

The Lucia™ reporter gene is provided in the pSELECT-zeo plasmid. The gene is flanked by two unique restriction sites to facilitate its subcloning. The pSELECT-zeo-Lucia plasmid can be used *in vivo* and *in vitro* to transfect mammalian cells stably or transiently. Lucia™ gene expression is driven by the constitutive EF-1 α /HTLV promoter that combines the elongation factor I α core promoter and the 5'untranslated region of the Human T-cell Leukemia Virus. The pSELECT-zeo-Lucia plasmid contains the zeocin resistance marker for selection in both mammalian cells and bacteria.

Contents and Storage

Lucia™ reporter gene is provided as 20 μ g of lyophilized DNA. It is supplied with 1 pouch of QUANTI-Luc™ and 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar; www.invivogen.com/fast-media). Plasmid is shipped at room temperature. Store at -20°C for up to one year.



PRODUCT	QTY	CAT. CODE
pSELECT-zeo-Lucia	20 μ g	psetz-lucia

Recombinant Lucia™ Protein

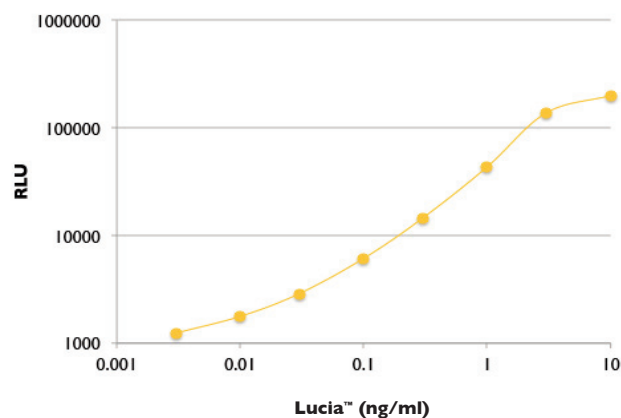
Recombinant Lucia™ Protein is a monomeric protein expressed in CHO cells. The mature protein is composed of 192 amino acids and has an estimated molecular weight of 21 kDa. Recombinant Lucia™ protein is provided in FBS-containing culture medium.

Application: Positive control for QUANTI-Luc™, a Lucia™ reporter assay reagent. A dilution series of the recombinant Lucia™ protein can also be used to determine the linear range of the assay.

Contents and Storage

Recombinant Lucia™ Protein is provided lyophilized. Product is shipped at room temperature. Store at -20°C for 12 months.

PRODUCT	QTY	CAT. CODE
Recombinant Lucia™ Protein	1 μ g	rec-lucia



Activity of recombinant Lucia™ protein determined by measuring Relative Light Units (RLU) in a luminometer using QUANTI-Luc™.

Anti-Lucia™ -IgG

Anti-Lucia™-IgG is a monoclonal mouse IgG1 antibody against Lucia™. It has been generated by immunizing mice with the recombinant Lucia™ protein and screened for neutralization activity. Anti-Lucia™-IgG is purified from the sera by Protein G affinity chromatography.

Application: Neutralization of Lucia™ used in a dual luciferase reporter assay employing the *Renilla* luciferase, another coelenterazine luciferase.

Contents and Storage

Anti-Lucia IgG is provided lyophilized from a 0.2 μ m filtered solution in PBS. Product is shipped at room temperature. Store at -20°C for 12 months.

PRODUCT	QTY	CAT. CODE
Anti-Lucia-IgG	100 μ g	mabg-lucia

QUANTI-Luc™

Lucia™ and coelenterazine-utilizing luciferases detection reagent (see last page)



Lucia™ Reporter Plasmids

pNiFty3-Lucia - Signal Transduction Reporter Plasmids

pNiFty3-Lucia is a family of inducible reporter plasmids that allow to monitor the activation of key signaling pathways. They feature the Lucia™ gene under the control of a synthetic promoter inducible by one or several defined transcription factors activated by these pathways. This promoter consists of the IFN-β minimal promoter combined with repeated transcription factor binding sites for nuclear factor (NF)-κB, activator protein I (AP-1), interferon regulatory factors (IRFs) and/or nuclear factor of activated T-cell (NFAT). pNiFty3-Lucia plasmids carry the Zeocin™ resistance gene for selection in mammalian cell lines and bacteria.

PLASMID NAME	TRANSCRIPTION FACTOR BINDING SITES	MINIMAL PROMOTER	SELECTION	REPORTER	CATALOG CODE
pNiFty3-Lucia	None	IFN-β	Zeocin™	Lucia™	pnf3-lc1
pNiFty3-N-Lucia	NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc2
pNiFty3-A-Lucia	AP-1 (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc3
pNiFty3-I-Lucia	ISRE (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc4
pNiFty3-T-Lucia	NFAT (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc5
pNiFty3-AN-Lucia	AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc6
pNiFty3-IAN-Lucia	ISRE (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc7
pNiFty3-TAN-Lucia	NFAT (x5) AP-1 (x5) NF-κB (x5)	IFN-β	Zeocin™	Lucia™	pnf3-lc8

Description

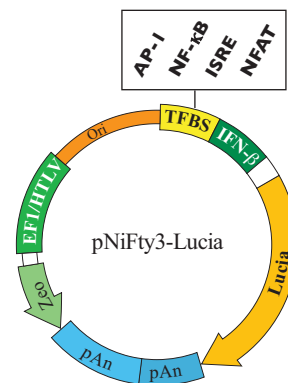
pNiFty3-Lucia plasmids are composed of three key elements: the mouse IFN-β minimal promoter, repeated transcription factor binding sites (TFBS) and the Lucia™ reporter gene.

- **NF-κB binding site:** Nuclear factor (NF)-κB is a “rapid-acting” primary transcription factor activated by a wide variety of PRRs. NF-κB is a protein complex that belongs to the Rel-homology domain-containing protein family. The prototypical NF-κB is composed of the p65(RelA) and p50 subunits. NF-κB binds specific decameric DNA sequences (GGGRNNYYCC, R-purine Y=pyrimidine) and activates genes involved in the regulation of the innate and adaptive immune response.

- **AP-1 binding site:** Activator protein I (AP-1) is a transcription factor activated by most PRRs. AP-1 is a heterodimeric complex composed of members of Fos, Jun and, ATF protein families. AP-1 binds to the TPA responsive element (TRE;TGAG/CTCA). AP-1 activation in TLR signaling is mostly mediated by MAP kinases such as c-Jun N-terminal kinase (JNK), p38 and extracellular signal regulated kinase (ERK).

- **ISRE binding site:** PRRs involved in the antiviral response induce the activation of interferon regulatory factors (IRFs) and the production of type I interferons (IFNs). IFNs trigger the formation of the ISGF3 complex which contains signal transducer and activator of transcription (STAT) 1, STAT2 and IRF9. ISGF3 and IRFs bind to specific nucleotide sequences called interferon-stimulated response elements (ISREs; AGTTTCNNTTCC) in the promoter of IFN-stimulated genes (ISGs) leading to their activation.

- **NFAT binding site:** Nuclear factor of activated T-cell (NFAT) is a family of transcription factors expressed in T cells, but also in other classes of immune and non-immune cells. NFAT is activated by stimulation of receptors coupled to calcium mobilization, such as the PRRs Dectin-1 and Mincle. Calcium mobilization induces the calmodulin-dependent phosphatase calcineurin leading to NFAT activation. NFAT binds to a 9 bp element, with the consensus sequence (A/T)GGAAA(A/N)(A/T/C)N.



Quality Control

HEK293 cells were transiently cotransfected with each pNiFty3-Lucia plasmid and a pUNO plasmid expressing the corresponding transcription factor (NF-κB/p65, AP-1/c-fos, ISRE/IRF7 or NFAT/NFATC2; see “Related Products”). After 48h, luciferase activity was measured using QUANTI-Luc™. Data available at www.invivogen.com/pnifty3

Contents and Storage

pNiFty3-Lucia plasmids are provided as lyophilized transformed *E. coli* strains on paper disk with 4 pouches of Fast-Media® Zeo (2 TB and 2 Agar). Products are shipped at room temperature and should be stored at -20°C.

Related Products

QUANTI-Luc™ and Zeocin™, see last page
 Fast-Media® Zeo, www.invivogen.com/fast-media
 pUNO-NFκBp65, pUNO-FOS, www.invivogen.com/gene.php
 pUNO-IRF7, pUNO-NFATC2, www.invivogen.com/gene.php

Lucia™ Reporter Cell Lines

InvivoGen has developed new reporter cell lines that feature the Lucia™ reporter gene. These cell lines allow the study of one or two signal transduction pathways following their activation by diverse molecules such as microbial ligands or cytokines. They were obtained by stable transfection of the extensively used human embryonic kidney (HEK) 293 cell line or well-established immune cell models, such as the human monocytic THP-1 cell line. Listed below are cell lines that express a dual reporter system containing the Lucia™ and SEAP (secreted embryonic alkaline phosphatase) reporter genes driven by either the same or two different inducible promoter constructs. Both reporters are readily measurable in the cell culture supernatant when using QUANTI-Luc™, a Lucia™ detection reagent, and QUANTI-Blue™, a SEAP detection reagent.

Dual™ Reporter Cells for the Detection of Bioactive Cytokines

HEK-Dual™ TNF-α Cells - TNF-α Lucia™/SEAP Reporter Cells

- Detection of bioactive human/mouse TNF-α
- Monitoring of NF-κB activation pathway

HEK-Dual™ TNF-α cells were specifically designed for the monitoring of bioactive TNF-α in biological samples by assessing NF-κB activation using either the Lucia™ or the SEAP reporter system.

HEK-Dual™ TNF-α cells were derived from the human embryonic kidney 293 cell line by stable co-transfection of two NF-κB-inducible reporter constructs. They feature the Lucia™ or SEAP reporter genes under the control of an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. Another feature of HEK-Dual™ TNF-α cells is their inability to respond to IL-1β due to a mutation in the IL-1β signaling pathway.

HEK-Dual™ TNF-α cells are resistant to Zeocin™.

Contents

HEK-Dual™ TNF-α cells are grown in DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin and 100 µg/ml Zeocin™. Cells are provided frozen in a cryotube containing 5-7 × 10⁶ cells. They are supplied with 50 mg of Normocin™, 10 mg of Zeocin™, 1 pouch of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™. Cells are shipped on dry ice.

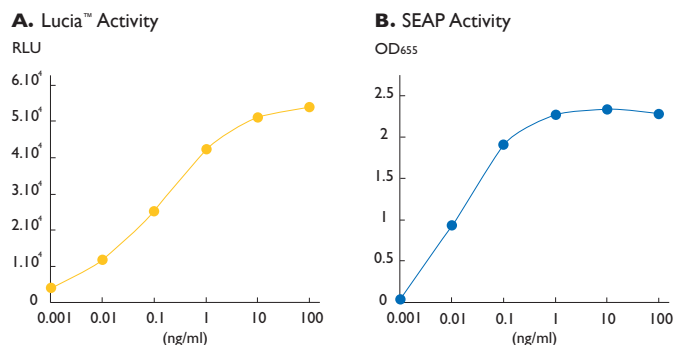
HEK-Dual™ IFN-γ Cells - IFN-γ Lucia™/SEAP Reporter Cells

- Detection of bioactive human IFN-γ
- Monitoring of JAK/STAT1 activation pathway

HEK-Dual™ IFN-γ cells allow the detection of bioactive human IFN-γ by monitoring the activation of the JAK/STAT-1 pathway using either the Lucia™ or the SEAP reporter system.

HEK-Blue™ IFN-γ cells were generated by stable transfection of HEK293 cells with the human STAT1 gene and two IFN-γ inducible reporter constructs featuring the Lucia™ or SEAP reporter gene under the control of an ISG54 promoter fused to four interferon-gamma-activated sites (GAS). HEK-Dual™ IFN-γ cells produce Lucia™ and SEAP in response to IFN-γ stimulation but are poorly responsive to type I and type III IFNs.

HEK-Dual™ IFN-γ cells are resistant to blasticidin, Zeocin™ and puromycin.



Lucia™ and SEAP Responses of HEK-Dual™ TNF-α cells to TNF-α. HEK-Dual™ TNF-α cells were incubated with increasing concentrations of recombinant human TNF-α. After 24h incubation, the levels of NF-κB-induced Lucia™ (A) and NF-κB-induced SEAP (B) were determined using QUANTI-Luc™ and QUANTI-Blue™, respectively. Lucia™ activity was determined by measuring Relative Light Units (RLU) while SEAP activity was assessed by measuring the OD at 655 nm.

- Detection range for human TNF-α: 0.001 - 10 ng/ml

PRODUCT	QUANTITY	CAT. CODE
HEK-Dual™ TNFα Cells	5-7 × 10 ⁶ cells	hkd-tnfa

Contents

HEK-Dual™ IFN-γ cells are grown in DMEM medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin, 30 µg/ml blasticidin, 100 µg/ml Zeocin™ and 1 µg/ml puromycin. Cells are provided frozen in a cryotube containing 5-7 × 10⁶ cells and supplied with 50 mg of Normocin™, 1 mg of blasticidin, 10 mg of Zeocin™, 1 mg of puromycin, 1 pouch of QUANTI-Blue™ and 1 pouch of QUANTI-Luc™. Cells are shipped on dry ice.

- Detection range for human IFN-γ: 1 - 10³ IU/ml

PRODUCT	QUANTITY	CAT. CODE
HEK-Dual™ IFN-γ cells	5-7 × 10 ⁶ cells	hkd-ifng

Dual™ Reporter Cells for the Study of the NF-κB and IFN Pathways

THPI-Dual™ (NF-κB - ISG) Cells - NF-κB-SEAP & IRF-Lucia Reporter Monocytes

THPI-Dual™ cells allow the simultaneous study of the NF-κB and interferon regulatory factor (IRF) pathways by monitoring the activity of SEAP and Lucia™, respectively.

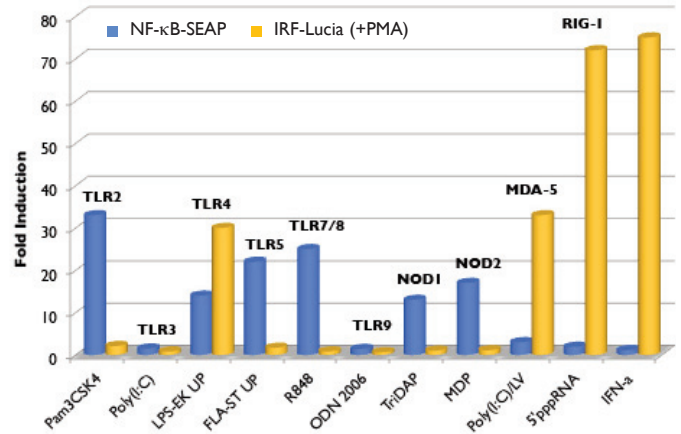
THPI-Dual™ cells were derived from the human THP-1 monocyte cell line by stable integration of two inducible reporter constructs. One contains the SEAP reporter gene under the control of an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. The second carries the Lucia™ gene driven by an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements.

THPI-Dual™ cells induce the activation of NF-κB in response to certain TLR agonists, such as Pam3CSK4 and flagellin. They trigger the IRF pathway upon stimulation with type I IFNs and RIG-I/MDA-5 agonists, such as transfected poly(I:C).

THPI-Dual™ cells are resistant to the selectable markers Zeocin™ and blasticidin.

Contents

THPI-Dual™ cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 μg/ml Zeocin™ and 10 μg/ml blasticidin. Cells are provided in a vial containing 5-7 × 10⁶ cells and supplied with 10 mg Zeocin™, 1 mg blasticidin, 1 pouch of QUANTI-Luc™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.



NF-κB/IRF dual Response of THPI-Dual™ cells. Cells were pretreated or not with PMA and incubated with 1 μg/ml Pam3CSK4, 1 μg/ml poly(I:C), 1 μg/ml LPS, 1 μg/ml flagellin, 10 μg/ml R848, 10 μg/ml ODN2006, 10 μg/ml Tri-DAP, 10 μg/ml MDP, 1 μg/ml poly(I:C)/LyoVec™, 1 μg/ml 5'pppRNA, or 10⁴ U/ml IFN-α. After 24h incubation, levels of SEAP and Lucia™ were assessed using QUANTI-Blue™ and QUANTI-Luc™, respectively.

PRODUCT	QUANTITY	CAT. CODE
THPI-Dual™ Cells	5-7 × 10 ⁶ cells	thpd-nfs

Jurkat-Dual™ (ISG - NF-κB) Cells - IRF-SEAP & NF-κB-Lucia Reporter T Lymphocytes

Jurkat-Dual™ cells allow the simultaneous study of the NF-κB and IRF pathways by monitoring the activity of Lucia™ and SEAP, respectively.

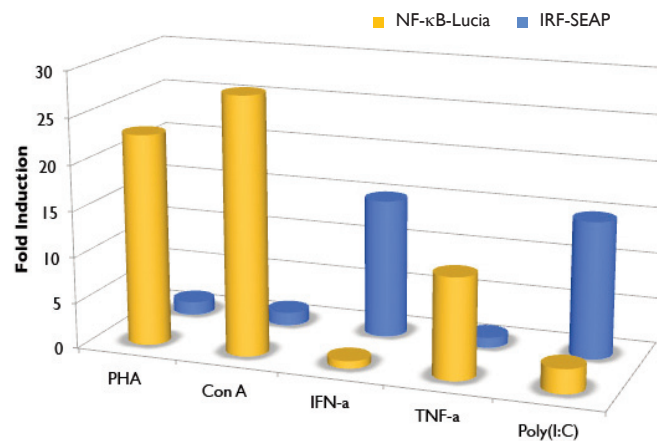
Jurkat-Dual™ cells were obtained by stable transfection of the human T lymphocyte-based Jurkat cell line with two inducible reporter constructs. The first construct features the Lucia™ gene driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. The second construct contains the SEAP gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements.

Jurkat-Dual™ cells induce the activation of NF-κB in response to TNF-α and T-lymphocyte mitogens, such as phytohemagglutinin and concanavalin A. They trigger the IRF pathway upon stimulation with type I IFNs and poly(I:C).

Jurkat-Dual™ cells are resistant to the selectable markers Zeocin™ and blasticidin.

Contents

Jurkat-Dual™ cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 μg/ml Zeocin™ and 10 μg/ml blasticidin. Cells are provided in a vial containing 5-7 × 10⁶ cells and supplied with 10 mg Zeocin™, 1 mg blasticidin, 1 pouch of QUANTI-Luc™ and 1 pouch of QUANTI-Blue™. Cells are shipped on dry ice. They are guaranteed mycoplasma-free.



NF-κB/IRF dual response of Jurkat-Dual™ cells. Cells were incubated with 50 μg/ml phytohemagglutinin (PHA), 50 μg/ml concanavalin A (Con A), 10⁴ IU/ml IFN-α, 10 ng/ml TNF-α or 10 μg/ml poly(I:C). After 24h incubation, the levels of NF-κB-induced Lucia and IRF-induced SEAP were assessed from the cell culture supernatant using QUANTI-Luc™ or QUANTI-Blue™, respectively.

PRODUCT	QUANTITY	CAT. CODE
Jurkat-Dual™ Cells	5-7 × 10 ⁶ cells	jktd-isnf

QUANTI-Luc™ - Coelenterazine-utilizing luciferase assay reagent

InvivoGen's new and original lyophilized product, QUANTI-Luc™, is an assay reagent containing all the components required to quantitatively measure the activity of Lucia™ and other coelenterazine-utilizing luciferases. Optimized for use with Lucia™ reporter cell lines for fast and efficient real-time measurements directly from the cell culture media. QUANTI-Luc™ contains the coelenterazine substrate for the luciferase reaction, which produces a light signal that is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

- **Ready to use** - Just add water
- **Cost effective** - One pouch prepares 5 × 96 well plates
- **Practical** - Working reagent stable at least one month

QUANTI-Luc™ is provided in a 2- or 5-pouch unit. Each pouch makes 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate. Product is shipped at room temperature. Store at -20°C up to 12 months. After preparation, product is stable 1 week at 4°C and 1 month at -20°C.

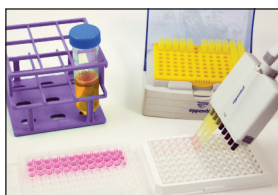
PRODUCT	QUANTITY*	CAT. CODE
QUANTI-Luc™	2 pouches (2 × 25 ml)	rep-qlc1
	5 pouches (5 × 25 ml)	rep-qlc2

* Bulk quantities readily available

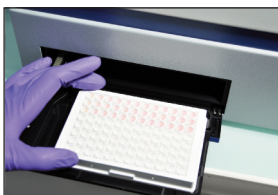
QUANTI-Luc™ Procedure



1. Prepare QUANTI-Luc by adding 25 ml water to the contents of one pouch.



2. Transfer aliquots of cell culture medium to opaque 96-microwell plate.



3. Set up the luminometer prior to addition of 50 µl QUANTI-Luc™ reagent to each well either manually or by automated injection. Measure luminosity in endpoint mode when using Lucia™ or in kinetic mode depending on the coelenterazine-luciferase used.

Selective Antibiotics

- The Highest Quality
- The Best Price
- Small Quantities to Bulk

InvivoGen is a leader in the production of selective antibiotics. We manufacture the largest choice of antibiotics for selection of stable mammalian cell lines. Our state-of-the-art facilities allow us to produce large quantities of high quality antibiotics at competitive prices. InvivoGen's selective antibiotics are ready-to-use, endotoxin and cell-culture tested solutions, available from small quantities to bulk. They are shipped at room temperature and should be stored at -20°C.

PRODUCT	QUANTITY	CAT. CODE
Blasticidin	100 mg (solution)	ant-bl-1
	500 mg (solution)	ant-bl-5
	1 g (powder)	ant-bl-10p
G418 Sulfate	1 g (solution)	ant-gn-1
	5 g (solution)	ant-gn-5
Hygromycin B	1 g (hygromycin B, solution)	ant-hm-1
	5 g (hygromycin B, solution)	ant-hm-5
HygroGold™	1 g (hygroGold™, solution)	ant-hg-1
	5 g (hygroGold™, solution)	ant-hg-5
	10 g (HygroGold™, powder)	ant-hg-10p
Puromycin	100 mg (solution)	ant-pr-1
	500 mg (solution)	ant-pr-5
Zeocin™	1 g (solution)	ant-zn-1
	5 g (solution)	ant-zn-5
	1 g (powder)	ant-zn-1p
	5 g (powder)	ant-zn-5p