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 hilgendorfi, formerly known as Cypridina hilgendorfi, of the Cypridinidae family. Later, secreted luciferases were isolated from luminous glands of marine copepod crustaceans, Gauussia princeps, Metridia longa, and Metridio pacifica of the Metridiidae family. Earlier this year, newly identified secreted luciferases were cloned from copepods of Heterorhabdidae, Lucicutiidae and Augapistiidae families, isolated from Zooplankton collected off the deep sea of Japan. 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 properties. 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 processes.

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

<table>
<thead>
<tr>
<th>Luciferase (Species)</th>
<th>Secreted</th>
<th>Size kDa</th>
<th>Emission type (T1/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Luciferin utilizing luciferases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photinus pyralis (firefly)</td>
<td>no</td>
<td>61</td>
<td>Glow</td>
</tr>
<tr>
<td>Vargula hilgendorfi</td>
<td>yes</td>
<td>62</td>
<td>Glow</td>
</tr>
<tr>
<td><strong>Coelenterazine utilizing luciferases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lucia™</td>
<td>yes</td>
<td>23</td>
<td>Extended flash (5 mins)</td>
</tr>
<tr>
<td>Renilla reniformis</td>
<td>no</td>
<td>36</td>
<td>Flash (30 sec)</td>
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<tr>
<td>Gauussia princeps</td>
<td>yes</td>
<td>20</td>
<td>Flash</td>
</tr>
<tr>
<td>Metridia longa</td>
<td>yes</td>
<td>24</td>
<td>Flash</td>
</tr>
<tr>
<td>Metridia pacifica</td>
<td>yes</td>
<td>20-23</td>
<td>Flash</td>
</tr>
</tbody>
</table>
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.

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-lasing, 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!

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InvivoGen 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

**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 Gaussia 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

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**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™.

**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-1α/HTLV promoter that combines the elongation factor 1 alpha 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 μg 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.

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 PBS-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.

<table>
<thead>
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<th>PRODUCT</th>
<th>QTY</th>
<th>CAT. CODE</th>
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<tbody>
<tr>
<td>Recombinant Lucia™ Protein</td>
<td>1 μg</td>
<td>rec-lucia</td>
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</tbody>
</table>

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 μm filtered solution in PBS. Product is shipped at room temperature. Store at -20°C for 12 months.

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

QUANTI-Luc™

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

For more information on InvivoGen’s products, visit www.invivogen.com
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 1 (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.

<table>
<thead>
<tr>
<th>PLASMID NAME</th>
<th>TRANSCRIPTION FACTOR BINDING SITES</th>
<th>MINIMAL PROMOTER</th>
<th>SELECTION</th>
<th>REPORTER</th>
<th>CATALOG CODE</th>
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<tbody>
<tr>
<td>pNiFty3-Lucia</td>
<td>None</td>
<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
<td>pnf3-lc1</td>
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<td>pNiFty3-N-Lucia</td>
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<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
<td>pnf3-lc2</td>
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<tr>
<td>pNiFty3-A-Lucia</td>
<td>AP-1 (x5)</td>
<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
<td>pnf3-lc3</td>
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<tr>
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<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
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<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
<td>pnf3-lc5</td>
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<td>pNiFty3-AN-Lucia</td>
<td>AP-1 (x5) NF-κB (x5)</td>
<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
<td>pnf3-lc6</td>
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<tr>
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<td>ISRE (x5) AP-1 (x5) NF-κB (x5)</td>
<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
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<td>IFN-β</td>
<td>Zeocin™</td>
<td>Lucia™</td>
<td>pnf3-lc8</td>
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</table>

**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 adaptative immune response.

- **AP-1 binding site**: Activator protein 1 (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; AGTTTCNNTTTCC) 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 -20ºC.

**Related Products**

QUANTI-Luc™ and Zeocin™, see last page
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™. 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/STAT1 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.

For more information on InvivoGen’s products, visit www.invivogen.com
**Dual™ Reporter Cells for the Study of the NF-κB and IFN Pathways**

**THP1-Dual™ (NF-κB - ISG) Cells - NF-κB-SEAP & IRF-Lucia Reporter Monocytes**

THP1-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.

THP1-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.

THP1-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).

THP1-Dual™ cells are resistant to the selectable markers Zeocin™ and blasticidin.

**Contents**

THP1-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 x 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 THP1-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.

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**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 x 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.

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**PRODUCT** | **QUANTITY** | **CAT. CODE**
--- | --- | ---
**THP1-Dual™ Cells** | 5-7 x 10⁶ cells | thpd-nfis

**PRODUCT** | **QUANTITY** | **CAT. CODE**
--- | --- | ---
**Jurkat-Dual™ Cells** | 5-7 x 10⁶ cells | jktd-nsnf
**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 quantitively 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 x 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.

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>QUANTITY*</th>
<th>CAT. CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUANTI-Luc™</td>
<td>2 pouches (2 x 25 ml)</td>
<td>rep-qlc1</td>
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<tr>
<td></td>
<td>5 pouches (5 x 25 ml)</td>
<td>rep-qlc2</td>
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</table>

* 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.

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**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.

<table>
<thead>
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