InvivoGen insight

MARCH 2018

SUMMARY :

REVIEW

Inflammasomes: connecting innate and adaptive immunity

PRODUCTS

Inflammasome reporter cells

- THP1-HMGB1-Lucia™ Cells
- THP1-ASC-GFP Cells

Inflammasome inhibitors

Dectin reporter cells

- HEK-Blue™ hDectin-1a Cells
- HEK-Blue™ hDectin-1b Cells
- HEK-Blue™ mDectin-1b Cells

Reporter detection reagents

- QUANTI-Luc™ Gold

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Inflammasomes : connecting innate and adaptive immunity

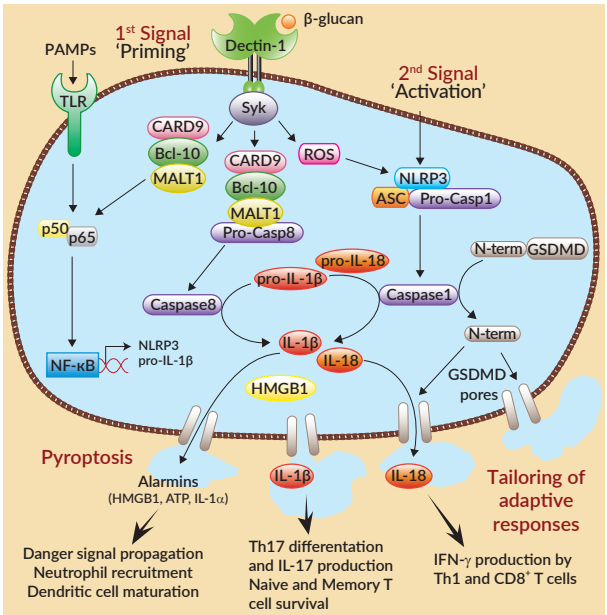
Fifteen years ago, the discovery of inflammasomes was a breakthrough in our comprehension of how inflammation is set off[1]. Inflammasomes have since been shown to play key roles in various pathophysiologic conditions and therefore constitute a major target for drug development. This review focuses on the central function of inflammasomes between innate and adaptive immunity.

Inflammasomes are tri-partite complexes consisting of a cytoplasmic sensor, an adaptor known as apoptosis-associated speck-like protein (ASC; or PYCARD), and pro-caspase-1. Inflammasomes are defined by their sensor, which include AIM2, Pyrin and NLRP1, NLRP3 and NLRC4 that belong to the NOD-like receptor family[2]. The diversity and specificity of sensors allow responsiveness to a broad range of stimuli either extrinsic (microbial molecules) or intrinsic (danger signals).

The NLRP3 inflammasome is the prototypical and best characterized inflammasome. Its activation is a two-step process. A first signal ('priming'), provided by microbial molecules such as lipopolysaccharide (LPS), induces NF-κB-dependent expression of NLRP3 and pro-IL1β. The second signal, provided by various structurally unrelated microbial molecules (e.g. toxins) or danger signals (e.g. monosodium urate (MSU)), triggers inflammasome multimerization. NLRP3 promotes ASC aggregation into specks leading to caspase-1 self-cleavage and activation. Active caspase-1 induces the proteolytic maturation of IL-1β and IL-18, and cleavage of Gasdermin D (GSDMD). Subsequent GSDMD pore formation at the cell membrane elicits a rapid pro-inflammatory form of cell death called pyroptosis. This event, associated with the release of IL-1β, IL-18 and alarmins such as HMGB1, contributes to the propagation of danger signals beyond the damaged or infected cell, notably by recruiting neutrophils[2,3]. Moreover, oligomeric inflammasome particles can be released to further amplify the inflammatory response after phagocytosis by surrounding macrophages[4].

Interestingly, IL-1β secretion can occur independently of pyroptosis in intact living phagocytes in a state of 'hyperactivation'[5,6]. Indeed, inflammasome-induced hyperactive dendritic cells (DCs) trigger enhanced T-cell responses: besides retaining their function of antigen presentation, they contextualize T helper cell responses through the secretion of IL-1β and IL-18. These cytokines were shown to preferentially drive Th1/Th17 responses. IL-18 amplifies IFN-γ production by primed Th1 cells and strengthen their Th1 differentiation, while IL-1β promotes Th17 polarization and IL-17 secretion. IL-1β also improves survival of naive and memory T cells[7,8]. Th17 responses driven by inflammasome-dependent IL-1β are essential for host defense against fungal infections. Recognition of fungal pathogens such as *Candida albicans* by Dectin-1, a C-type lectin receptor that senses β-glucans, leads to Syk-dependent NF-κB activation, NLRP3 inflammasome assembly and IL-1β-mediated Th17 responses resulting in effective anti-*Candida* immunity[9,10]. Of note, Dectin-1 signaling also triggers IL-1β production via a non-canonical caspase-8 inflammasome[9].

The divergent functions of IL-1β and IL-18 in shaping adaptive immunity has drawn much attention to inflammasomes for vaccine adjuvant development. Th1-mediated humoral responses, Th1/Th17/ cytotoxic T cell immunity and immunological memory may be manipulated with inflammasome-activating ligands[11]. For example, combining induction of type-I interferons (IFNs), known to inhibit pro-IL-1 synthesis and favor IL-18 maturation, with activation of inflammasomes in DCs is a promising way to achieve protective Th1 responses[11]. Such combination may be obtained with certain particulate vaccine adjuvants such as the cationic polysaccharide chitosan. It was recently found to induce type-I IFN production and NLRP3 inflammasome activation leading to robust Th1 responses[13]. Further studies are still needed to better understand the role of inflammasomes in adaptive immunity and for the development of improved vaccine adjuvants.



1. Martinon F. *et al.*, 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1β. Mol Cell. 10:417-26. **2. Broz P. and Dixit V.M., 2016.** Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol. 16:407-20. **3. Kovacs S.B. and Miao E.A., 2017.** Gasdermins: effectors of pyroptosis. Trends Cell Biol. 27:673-84. **4. Baroja-Mazo A. *et al.*, 2014.** The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. Nat. Immunol. 15:738-48. **5. Broz P. *et al.*, 2010.** Differential requirement for caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. Cell Host Microbe. 8:471-83. **6. Evavold C.L. *et al.*, 2018.** The pore-forming protein gasdermin D regulates interleukin-1 secretion from living macrophages. Immunity. 48:1-10. **7. Evavold C.L. and Kagan J.C., 2018.** How inflammasomes inform adaptive immunity. J Mol Biol. 430:217-37. **8. Zhu J. *et al.*, 2010.** Differentiation of effector CD4 T cell populations. Annu Rev Immunol. 28:445-489. **9. Tang J. *et al.*, 2018.** Regulation of C-type lectin receptor-mediated antifungal immunity. Front. Immunol. 9:123. **10. Geijtenbeek T.B.H. and Gringhuis S.I., 2016.** C-type lectin receptors in the control of T helper cell differentiation. Nat Rev Immunol. 16:433-48. **11. Muñoz-Wolf N. *et al.*, 2017.** The role of inflammasomes in adjuvant-driven humoral and cellular immune responses. Immunopotentiators in Modern Vaccines. 23-42. **12. Dostert C. *et al.* 2013.** Innate and adaptive effects of inflammasomes on T cell responses. Curr. Opin. Immunol. 25:359-65. **13. Carroll E.C. *et al.*, 2016.** The vaccine adjuvant chitosan promotes cellular immunity via DNA sensor cGAS-STING-dependent induction of type I interferons. Immunity. 44:597-608.

Inflammasome Reporter Cells

InvivoGen has developed two THP-1-derived reporter cells to facilitate inflammasome studies. THP-1 is a monocytic cell line and the most commonly used human model for the study of inflammasome activation *in vitro*. It requires a priming step prior to stimulation with an inflammasome inducer for optimal release of IL-1β. Our THP-1-derived inflammasome reporter cells are designed to study pyroptosis and ASC speck formation.

THP1-HMGB1-Lucia™ Cells NEW

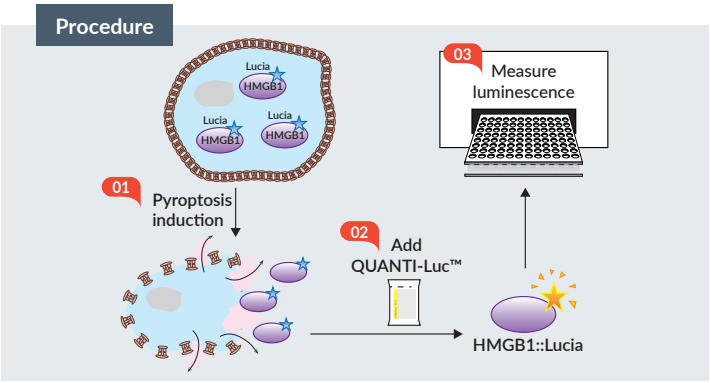
THP1-HMGB1-Lucia™ cells represent a unique tool for robust and convenient quantification of pyroptosis. This assay is an alternative to the classical lactate deshydrogenase (LDH) assay, and relies on the measurement of cytolysis-mediated release of HMGB1, the prototypic alarmin, fused to Lucia luciferase.

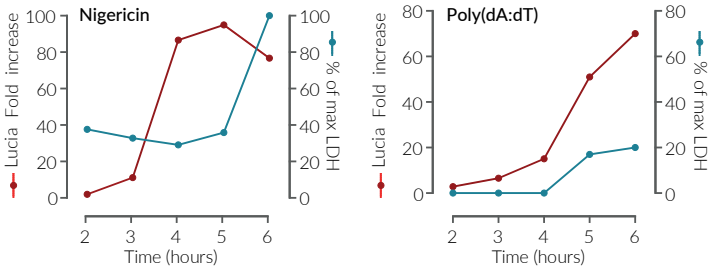
- **Rapid:** mix supernatant + detection reagent and measure luminescence
- **Sensitive:** low background and better range of detection than LDH assays
- **Convenient:** only 10 µl of supernatant required – allows kinetics
- **Flexible:** enables pyroptosis and IL-1β release evaluation

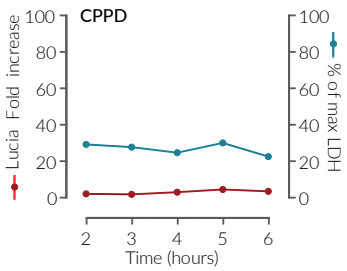
THP1-HMGB1-Lucia™ cells are typically primed with LPS before treatment with inflammasome inducers, such as Nigericin and CPPD crystals (NLRP3 inducers) or poly(dA:dT) (AIM2 inducer). Inflammasome activation leads to pyroptosis-mediated release of HMGB1::Lucia in the extracellular milieu. Levels of HMGB1::Lucia in the supernatant can be readily monitored by measuring the light signal produced after addition of QUANTI-Luc™ which provides the coelenterazine substrate.

HMGB1::Lucia cell supernatants can also be assessed for IL-1β activity by using the IL-1β reporter cell line, HEK-Blue™ IL-1β. This cell line carries an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene which expression can be monitored using QUANTI-Blue™.

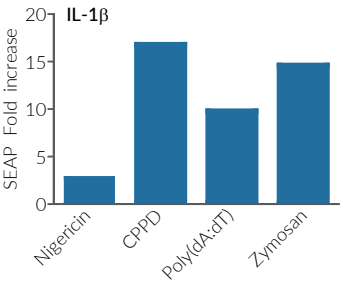
Inflammasome inducers may differentially trigger pyroptosis and IL-1β secretion: Nigericin is a rapid and potent inducer of pyroptosis but a weak producer of IL-1β, in contrast to CPPD crystals which are unable to trigger pyroptosis but induce high levels of IL-1β. Transfection with Poly(dA:dT) strongly induces both pyroptosis and IL-1β.







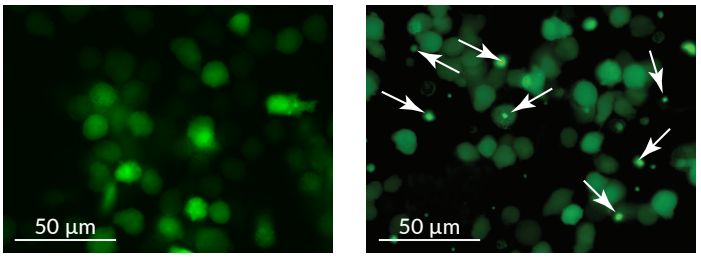
Pyroptosis response of THP1-HMGB1-Lucia™ cells: cells were primed with 1 µg/ml LPS-EK for 3h and then incubated with inflammasome inducers (8 µM Nigericin, 0.5 µg/ml complexed Poly(dA:dT) or 100 µg/ml CPPD). Lucia luciferase activity and LDH release in the supernatant were quantified at 2, 3, 4, 5 and 6 hours post-induction.



NF-κB response of HEK-Blue™ IL-1β cells: THP1-HMGB1-Lucia™ cells were incubated with inflammasome inducers (8µM Nigericin, 100 µg/ml CPPD, 0.5 µg/ml complexed Poly(dA:dT) or 1 mg/ml Zymozan). After 24h, supernatants were incubated with HEK-Blue™ IL-1β cells overnight and SEAP activity was assessed using QUANTI-Blue™.

THP1-ASC-GFP Cells

THP1 ASC-GFP cells stably express an ASC::GFP fusion protein that enables the visual monitoring of large ASC protein complexes termed "specks". ASC::GFP expression is driven by an NF-κB-inducible promoter and is induced upon LPS priming. Subsequent stimulation with compounds such as Poly(dA:dT) results in inflammasome activation which can be analyzed by visualization of fluorescent ASC speck formation.



Visualization of ASC speck formation by fluorescence microscopy: THP1-ASC-GFP cells were primed with 1 µg/ml LPS-EK for 3 hours, inducing the expression of the ASC::GFP fusion protein in the cytoplasm (left panel). Cells were then incubated with 250 ng/ml complexed Poly(dA:dT) and ASC speck formation was monitored over 1 to 3 hours post-activation. In most cells, only one speck forms upon inflammasome activation (arrows in right pannel). Scale bar: 50 µm.

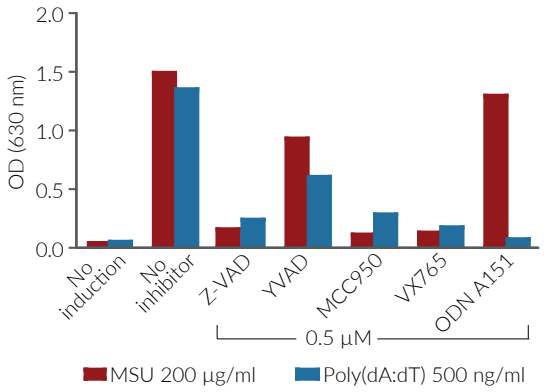
PRODUCT	QUANTITY	CAT. CODE
THP1-HMGB1-Lucia™ cells	3-7 x 10^6 cells	thp-hmgluc
THP1-ASC-GFP cells	3-7 x 10^6 cells	thp-ascgfp
HEK-Blue™ IL-1β cells	3-7 x 10^6 cells	hkb-il1b
CPPD	5 mg	tlrl-cppd
Nigericin	10 mg	tlrl-nig
Poly(dA:dT)	200 µg	tlrl-patn

www.invivogen.com/inflammasome-test-cells

Inflammasome inhibitors

InvivoGen offers a selection of inhibitors known to block inflammasome-induced immune responses. These inhibitors act on different targets such as NLRP3, AIM2 or caspase-1. They have been functionally validated using THP1-HMGB1-Lucia™ cells to activate the inflammasome and HEK-Blue™ IL-1β cells to measure the release of bioactive IL-1β. Furthermore, our inflammasome inhibitors are tested to confirm the absence of bacterial contaminants thus preventing experimental bias.

PRODUCT	MAIN TARGET	QUANTITY	CAT. CODE
Ac-YVAD-cmk	Caspase-1	5 mg	inh-yvad
MCC950	NLRP3	10 mg	inh-mcc
ODN TTAGGG (A151)	AIM2	200 µg	tlrl-ttag151
VX-765	Caspase-1	10 mg	inh-vx765i-1
Z-VAD-FMK	Pan-caspase	1 mg	tlrl-vad



Effect of inflammasome inhibitors on IL-1β release by THP1-HMGB1-Lucia™ cells: THP1-HMGB1-Lucia™ cells were primed with LPS and treated with MSU (200 µg/ml) or Poly(dA:dT) complexes (0.5 µg/ml) in the presence of various inhibitors at 0.5 µM. After overnight incubation, cell supernatants were added to HEK-Blue™ IL-1β cells for 16 hours. IL-1β levels were determined by assessing SEAP activity in the supernatant using QUANTI-Blue™.

www.invivogen.com/inflammasome-inhibitors

Dectin-1a and Dectin-1b reporter cells

InvivoGen provides a new series of Dectin-1 reporter cells derived from the human embryonic kidney HEK293 cell line. Dectin-1 is a C-type lectin receptor (CLR) that recognizes β-glucans, glucose polymers found in the cell walls of fungi, and thus plays an important role in antifungal innate immunity.

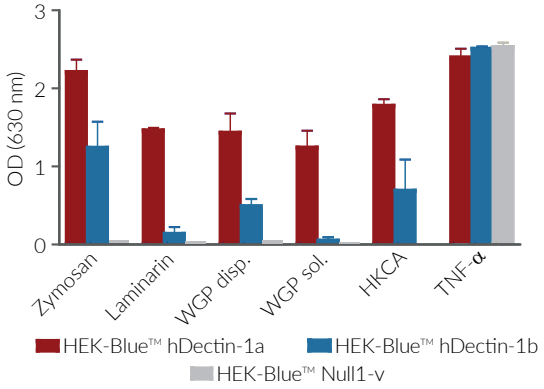
HEK-Blue™ hDectin-1a Cells NEW

HEK-Blue™ hDectin-1b Cells

HEK-Blue™ mDectin-1b Cells NEW

Dectin-1 is alternatively spliced into 2 major isoforms: a full-length A isoform and a 'stalkless' B isoform, which do not induce the same response to soluble and particulate β-glucans[1]. Contrarily to mouse RAW 264.7 macrophages which endogenously express both Dectin-1 isoforms at low protein level, HEK293 cells do not express Dectin-1. Therefore, we have engineered HEK-Blue cells that stably express high levels of either human dectin-1a or -1b isoform and an NF-κB -inducible secreted alkaline phosphatase (SEAP). These cells also express genes involved in the Dectin-1 signaling pathway leading to NF-κB activation.

PRODUCT	QUANTITY	CAT. CODE
HEK-Blue™ hDectin-1a cells	3-7 x 10^6 cells	hkb-hdect1a
HEK-Blue™ hDectin-1b cells	3-7 x 10^6 cells	hkb-hdect1b
HEK-Blue™ mDectin-1b cells	3-7 x 10^6 cells	hkb-mdect1b
HEK-Blue™ Null I-v cells (control)	3-7 x 10^6 cells	hkb-null1v
QUANTI-Blue™ (5 pouches)	5 x 100 ml	rep-qb1
Zymozan (*S. cerevisiae*)	100 mg	tlrl-zyn
Laminarin (*L. digitata*)	100 mg	tlrl-lam
WGP dispersible (*S. cerevisiae*)	50 mg	tlrl-wgp
WGP soluble (*S. cerevisiae*)	50 mg	tlrl-wgps
HKCA (*C. albicans*)	10^9 cells	tlrl-hkca



NF-κB responses of HEK-Blue™ hDectin-1a and -1b and HEK-Blue™ Null I-v cells to Dectin-1 agonists: 10 µg/ml Zymozan, Laminarin, WGP soluble, 100 µg/ml WGP dispersible, or 3 x 10^6 cells/ml HKCA. 10 ng/ml TNF-α was used as a positive control. After 24h, SEAP activity was assessed in the supernatant using QUANTI-Blue™.

While HEK-Blue™ hDectin-1a cells respond well to both particulate and soluble ligands, HEK-Blue™ hDectin-1b cells display a reduced response to particulate ligands and a weak response to soluble ligands. This is in line with the long-disputed theory of the antagonistic activity of soluble Dectin-1 ligands such as laminarin. In addition, these results further support a recent report showing that the size and purity of soluble ligands dictate their agonist/antagonist activity[2]. Our cell lines allow the determination of the biological activity of soluble and particulate compounds in a specific manner.

1. Heinsbroek, S.E, et al, 2006. Expression of functionally different Dectin-1 isoforms by murine macrophages. J Immunol. 176: 5513. **2. Smith A.J., et al, 2018.** Immunoregulatory activity of the natural product Laminarin varies widely as a result of its physicial properties. J Immunol. 200:788.

www.invivogen.com/hek-blue-clr

Reporter Detection Reagents

QUANTI-Luc™ Gold NEW

Optimized Lucia and coelenterazine luciferases detection assay

- **Two-component reporter kit:** QUANTI-Luc™ Plus and QLC Stabilizer
- **Two options:** use QUANTI-Luc Plus
  - without QLC Stabilizer for enhanced luminescent signal
  - with QLC Stabilizer for enhanced signal stability over 10 minutes
- **Ideal for high-throughput screening**
- **Usable with cell supernatant or directly with cells**

QUANTI-Luc™ Gold is a new reporter detection kit that can be used to detect the activity of coelenterazine-utilizing luciferases (e.g. Lucia, Gaussia and Renilla luciferases). It comprises **QUANTI-Luc™ Plus**, an optimized coelenterazine-containing reagent, and **QLC Stabilizer**. This kit enables flexible luciferase activity measurement:

(a) QUANTI-Luc™ Plus without QLC stabilizer allows enhanced light output detection (as compared to our standard QUANTI-Luc™).

(b) QUANTI-Luc™ Plus with QLC stabilizer allows enhanced light signal stability (over 10 minutes). This option is ideal for high-throughput screening or time-course studies.

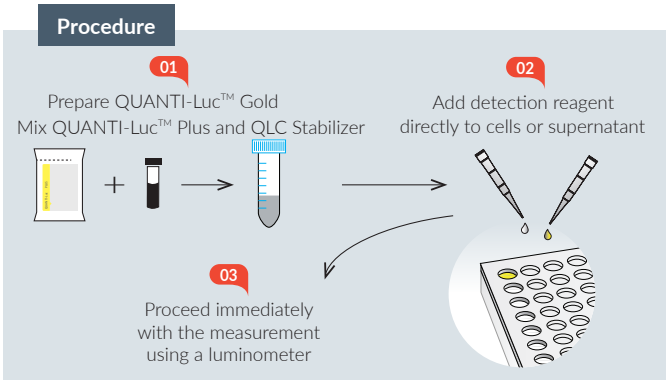
The signal revealed by QUANTI-Luc™ Gold is quantified using a luminometer and expressed as relative light units (RLUs).

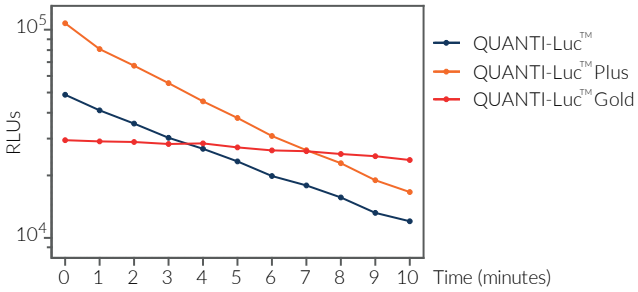
1 pouch:
5 x 96-well plates or 2 x 1536-well plates

2 pouches:
10 x 96-well plates or 5 x 1536-well plates

5 pouches:
25 x 96-well plates or 13 x 1536-well plates

www.invivogen.com/reporter-detection





Lucia luciferase activity detection with QUANTI-Luc-based detection media: THP1-Dual™ cells were stimulated for 24h with 10 µg/ml 2'3'-cGAMP. The ISG response was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™, QUANTI-Luc™ Plus, or QUANTI-Luc™ Gold (QUANTI-Luc™ Plus with QLC Stabilizer). Relative light units (RLUs) were measured over 10 minutes.

PRODUCT	QUANTITY	CAT. CODE
QUANTI-Luc™ Gold	1 pouch (25 ml) 2 pouches (50 ml) 5 pouches (125 ml) 1 pouch (500 ml)	rep-qlcg1 rep-qlcg2 rep-qlcg5 rep-qlcg-500
QUANTI-Luc™	2 pouches (50 ml) 5 pouches (125 ml) 1 pouch (500 ml)	rep-qlc1 rep-qlc2 rep-qlc-500

Selective Antibiotics

Cell-culture tested antibiotics

- **Sterile**
- **Endotoxin-free**
- **Functionally tested**

InvivoGen offers a range of cell-culture tested antibiotics to ensure artifact-free selection of transfected mammalian cells. These antibiotics are sterile and endotoxin-free to avoid the deleterious effects of bacterial endotoxins on transfected cells. They are functionally validated through rigorous physico-chemical, microbiological and cellular testing. They exhibit proven long-term stability to mammalian cells with no cytotoxicity.

PRODUCT	QUANTITY	CAT. CODE
Blasticidin	100 mg (10 x 1 ml)	ant-bl-1
G418 (Geneticin)	1 g (10 x 1 ml)	ant-gn-1
Hygromycin B Gold	1 g (10 x 1 ml)	ant-hg-1
Puromycin	100 mg (10 x 1 ml)	ant-pr-1
Zeocin™	1 g (10 x 1 ml)	ant-zn-1

www.invivogen.com/selective-antibiotics

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