InvivoGen insight

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InvivoGen
www.invivogen.com
2025

Metaflammation: We Are What We Eat

The consumption of Western diets — high in sugar and saturated fat, combined with sedentary behavior — has led to an epidemic of lifestyle-associated, non-communicable diseases, including type 2 diabetes, obesity, and cardiovascular conditions[1]. A growing body of evidence has linked these health issues to a 'silent' inflammation, whose conceptualization and underpinning mechanisms are described in the metaflammation theory[2,3].

Rethinking inflammation

Metaflammation is a term first introduced in 2006 to describe a type of chronic, low-grade inflammation observed in metabolically impactful tissues, such as adipose tissue, pancreatic islets, the liver, and even the brain, causing severe systemic damage over time, which leads to disrupted metabolic pathways and pathogenesis[2]. The discovery of metabolically triggered inflammation has led to a paradigm shift from the traditional view of inflammation as an acute, short-term response to infection or external injury[2].

Adipose tissue: the usual suspect

For a long time, excess adipose tissue was seen as the primary driver of metaflammation[4]. As fat mass expands, the fat-storing cells, known as adipocytes, suffer hypoxia and mechanical stress. The overloaded adipose tissue loses its ability to store excess fat efficiently, causing the formation of danger-associated molecular patterns (DAMPs). Reactive oxidative species (ROS) and lipid byproducts, like ceramides and cholesterol from free fatty acids, are released into the bloodstream, further contributing to systemic inflammation[4,5]. Moreover, the stressed adipocytes secrete pro-inflammatory molecules, such as adipokines and cytokines (e.g. leptin, TNF-α, IL-1β) to recruit macrophages into the adipose tissue, where they shift to a pro-inflammatory state[4,5]. Over time, this localized 'sterile' inflammation spills over into systemic circulation, contributing to metabolic dysregulation (e.g. insulin resistance) and disease onset[4,5]. In short, obesity was thought to be the predominant, and linear cause of metaflammation. However, as research has advanced, it has become clear that while adipose tissue plays an essential role in metaflammation, it is only one part of a much more complex network[4].

The gut-brain axis in metaflammation

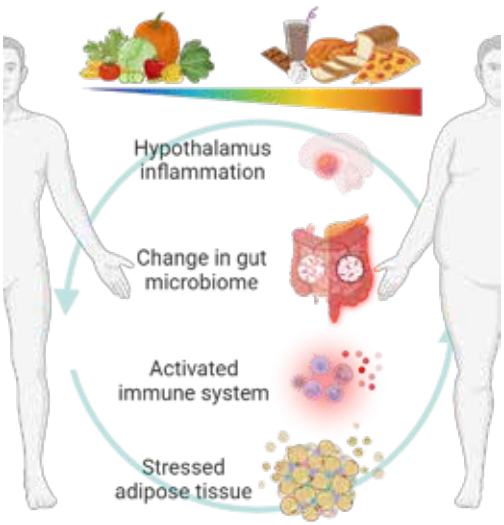
Recent studies have shown that metaflammation in the brain, particularly in the **hypothalamus**, can occur early in the process of obesity development, often before significant fat accumulation[6]. This observation raised the question of whether hypothalamic inflammation could actually be the cause of obesity rather than a consequence[6]. The hypothalamus is the most important regulatory center in the brain, controlling hunger, satiety, and energy expenditure via hormones, such as leptin and insulin[6]. It has been shown that even a short exposure to high-fat, high-sugar diets can trigger reactive gliosis, a process involving the chronic activation of microglia and astrocytes in the brain. This leads to the release of pro-inflammatory cytokines, promoting metaflammation and weakening of the blood-brain-barrier[6]. Consequently, dietary fats easily accumulate in the hypothalamus, impairing insulin and leptin signaling pathways, which in turn causes dysregulated appetite control and weight gain due to increased food consumption[6].

> Metaflammation describes a chronic, low-grade inflammation.

The constant excess of calorie-dense food is shown to adversely alter the composition of the gut microbiota, increasing intestinal permeability and thereby allowing bacterial endotoxins, also known as lipopolysaccharides (LPS), to enter the bloodstream[1,7] - further fueling this **inflammatory cycle.**

The real culprits: TLR4 & NLRP3

LPS is a well-known pathogen-associated molecular pattern and is recognized by Toll-like receptor 4 (TLR4), one of the pattern recognition receptors (PRRs) associated with metaflammation[8]. Activation of TLR4 by LPS, free fatty acids, and ceramides was found to disrupt leptin[6,9] and insulin[6] signaling through inflammatory pathways, including IKKβ, NF-κB, and JNK, as well as NLRP3 (NOD-like receptor family, pyrin domain containing 3) inflammasome priming[5]. Enhanced IKKβ/NF-κB signaling in the hypothalamus interferes with the leptin pathway by affecting STAT3 phosphorylation and upregulating SOCS3, a negative regulator of leptin signaling[9]. The activation of NLRP3, a key amplifier of inflammatory responses, allows for a rapid response to the aforementioned DAMPs, ROS and cholesterol crystals. Additionally, other 'sterile' DAMPs, such as beta-amyloids, ATP, and monosodium urate crystals, can trigger the **NLRP3 inflammasome**, resulting in IL-1β secretion as well as pyroptotic cell death[5,11].



Excessive IL-1β production has been demonstrated to play a key role in obesity-induced diabetes by altering the insulin pathway. It reduces the translocation of the glucose transporter type 4 and inhibits insulin receptor functions, ultimately decreasing glucose uptake and disrupting insulin sensitivity in peripheral tissues, which promotes insulin resistance[10]. Notably, blocking NLRP3 activation has been found to improve insulin sensitivity[13].

Taming NLRP3 is key

Thus, NLRP3 holds great potential as a drug target for a range of diseases, including obesity, neurodegenerative, and cardiovascular conditions[5]. Indeed, NLRP3 knockout and prophylactic dosing with the NLRP3 inhibitor MCC950 (CP-456773) protected animals against diet-induced obesity[12]. Although the precise mechanisms linking NLRP3 inhibition and reduced metaflammation to weight loss are not fully understood, it has been suggested that these effects mainly result from lowering the chronic inflammation in the hypothalamus[12]. Reduced central nervous system (CNS) inflammation has been shown to restore insulin and leptin sensitivity[6]. This improvement leads to normalized eating behaviors, a decrease in calorie intake, and subsequently, a healthy microbiota composition and weight loss.

A promising new drug NT-0796

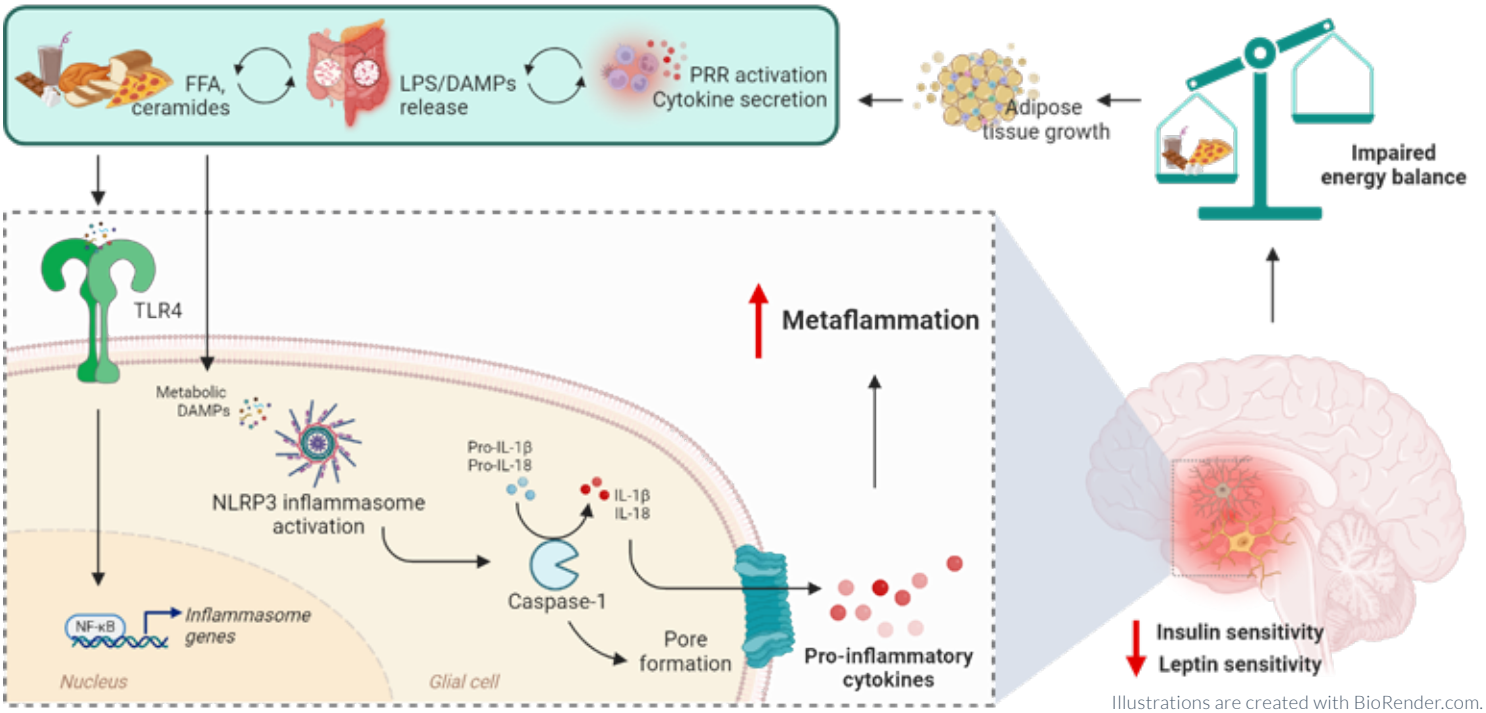
Interestingly, preestablished obesity was reversed using NT-0796, a **novel clinical-stage NLRP3 inhibitor**[12]. NT-0796 is orally available, exhibits good systemic distribution, and can cross the blood–brain barrier, opening the possibility to treat a number of conditions associated with chronic inflammation of the periphery or CNS[12].

> **NLRP3 inhibition can prevent and reverse diet-induced obesity.**

Initially developed by NodThera as a lead clinical-stage therapy for Parkinson's disease, NT-0796 has also demonstrated nearly equivalent effectiveness, in promoting weight loss, to Novo Nordisk's blockbuster GLP-1 receptor agonist semaglutide, marketed as Wegovy®[12]. Moreover, NT-0796 treatment reduces the expression of disease-relevant metabolic and cardiovascular inflammatory biomarkers like fibrinogen, VCAM-1 (vascular cell adhesion protein 1), and PCSK9 (proprotein convertase subtilisin/kexin type 9)[12]. It also decreases levels of GFAP (glial fibrillary acidic protein), a key marker of reactive gliosis in the brain[12].

Uncovering that NLRP3 and other PRRs do not only detect pathogens but are also crucial in driving metaflammation due to nutrient sensing has unveiled exciting new targets. Beyond innovative drugs that curb pro-inflammatory PRR activation, lifestyle changes like caloric restriction and balanced diets represent promising candidates to counteract metaflammation and its detrimental effects on our health[11,12]. Overall, we are what we eat.

1. Christ A, *et al.*, 2019. Immunity. 51(5):794-811. **2. Hotamisligil GS, 2006.** Nature. 14;444(7121):860-7. **3. Franceschi C, *et al.*, 2017.** Trends Endocrinol Metab.;28(3):199-212. **4. Lumeng CN, Saltiel AR. 2011.** J Clin Invest. 121(6):2111-7. **5. Ramachandran R, *et al.*, 2024.** Exp Mol Med. ;56(7):1488-1500. **6. Sonnefeld L, *et al.*, 2023.** Eur J Endocrinol. 188(3):R37-R45. **7. Cani PD, *et al.*, 2007.** Diabetes. 56(7):1761-72. **8. Saltiel AR, Olefsky JM, 2017.** J Clin Invest. 127(1):1-4. **9. de Git KC, Adan RA,. 2015.** Obes Rev. 16(3):207-24. **10. Jager J, *et al.*, 2007.** Endocrinology. 148(1):241-51. **11. Harrison D, *et al.*, 2023.** J Med Chem. 66(21):14897-14911 **12. Thornton *et al.*, 2024.** J Pharmacol Exp Ther. 388(3):813-826. **13. Vandanmagsar B, *et al.*, 2011.** Nat Med. (2):179-88.



Illustrations are created with BioRender.com.

Diet-induced activation of the NLRP3 inflammasome drives hypothalamic metaflammation and obesity onset. Components of a Western diet, particularly free fatty acids (FFAs) and ceramides, trigger NLRP3 inflammasome activation through Toll-Like Receptor 4 (TLR4). In parallel, lipopolysaccharides (LPS) and other danger-associated molecular patterns (DAMPs) released from the altered gut microbiota into the bloodstream accumulate in the brain. In the hypothalamus, the TLR4-mediated NLRP3 activation induces the processing of pro-inflammatory cytokines IL-1β and IL-18 via caspase-1. Caspase-1 also triggers pore formation in the cell membrane, facilitating the secretion of pro-inflammatory cytokines. This inflammatory cascade chronically activates glial cells, contributing to insulin and leptin resistance, which disrupts appetite control and energy balance. The impaired signaling promotes increased food intake and, consequently, adipose tissue accumulation. The overloaded adipose tissue loses its ability to store excess fat efficiently, releases DAMPs, and further fuels this vicious cycle of metaflammation.

NLRP3 inflammasome inducer

InvivoGen offers ready-to-use Cholesterol crystals, a potent inducer of the NLRP3 inflammasome. These crystals are thoroughly tested to ensure reproducible and reliable results.

• Cholesterol crystals NEW

Cholesterol crystals are the hallmark of atherosclerosis; a condition where plaque builds up in arteries, increasing the risk of heart attacks and strokes[1]. They act as key danger-associated molecular patterns (DAMPs) able to stimulate the NLRP3 inflammasome-dependent induction of pro-inflammatory cytokines, namely interleukin 1β (IL-1β) and IL-18[1].

InvivoGen provides Cholesterol crystals prepared by precipitation of ultrapure cholesterol in 1-propanol in a controlled environment. Each lot features similar shape and size and is tested *in vitro* using a bioassay based on THP1-Null2 cells and HEK-Blue™ IL-1β cells *(Fig. 1)*.

PRODUCTS	QTY	CAT. CODE
Cholesterol crystals	20 mg	tlrl-chol

RELATED PRODUCTS

- **THP1-Null2:** Human IL-1β reporter cells (thp-nullz)
- **HEK-Blue™ IL-1β:** Human IL-1β reporter cells (hkb-il1bv2)

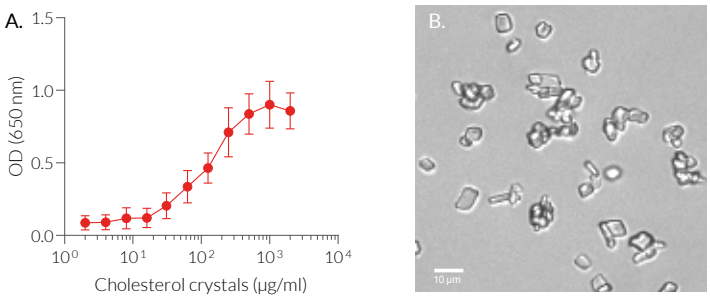


Figure 1A. IL-1β production in THP1-Null cells upon Cholesterol crystal-induced NLRP3 activation. Human THP-1 monocytes (primed with LPS-EK) were incubated with increasing concentrations of Cholesterol crystals. After 24 h, the IL-1β secretion was assessed using HEK-Blue™ IL-1β sensor cells and the SEAP detection reagent QUANTI-Blue™ Solution. Data is shown as optical density (OD) (mean ± SD). **B. Microscopic analysis.** Cholesterol crystals resuspended in sterile water.

Learn more here www.invivogen.com/inflammasome-inducers

1. Duewell P, *et al.*, 2010. Nature. 464(7293):1357-611.

NLRP3 inflammasome inhibitor

InvivoGen provides NT-0796, a selective, oral and brain-penetrant inhibitor of the NLRP3 inflammasome that is more potent than MCC950 for the blockade of NLRP3-induced inflammation.

• NT-0796 NEW

The **NT-0796** is an ester prodrug which is converted by human carboxylesterase-1 (CES1) to the active metabolite NDT-19795, a specific inhibitor of the NLRP3 inflammasome[1]. Human macrophages are highly sensitive to NT-0796, unlike mouse macrophages that lack CES1. NT-0796 is showing promising results in clinical trials for the treatment of chronic inflammatory diseases.

InvivoGen's NT-0796 is provided in the InvitroFit™ grade:
- Functionally tested
- Highly pure (≥95%)
- Endotoxin-free

NT-0796 inhibits NLRP3-dependent cell death more potently than MCC950, as verified using the cytotoxic assay LDH-Blue™ *(Fig.1)*.

PRODUCTS	QTY	CAT. CODE
NT-0796	1 mg	inh-nt0796

RELATED PRODUCTS

- **LDH-Blue™:** LDH activity assay kit (rep-ldh-1)
- **MCC950:** NLRP3 inhibitor - InvitroFit™ (inh-mcc)
- **MSU crystals:** NLRP3 inducer (tlrl-msu)
- **Nigericin:** NLRP3 inducer (tlrl-nig)

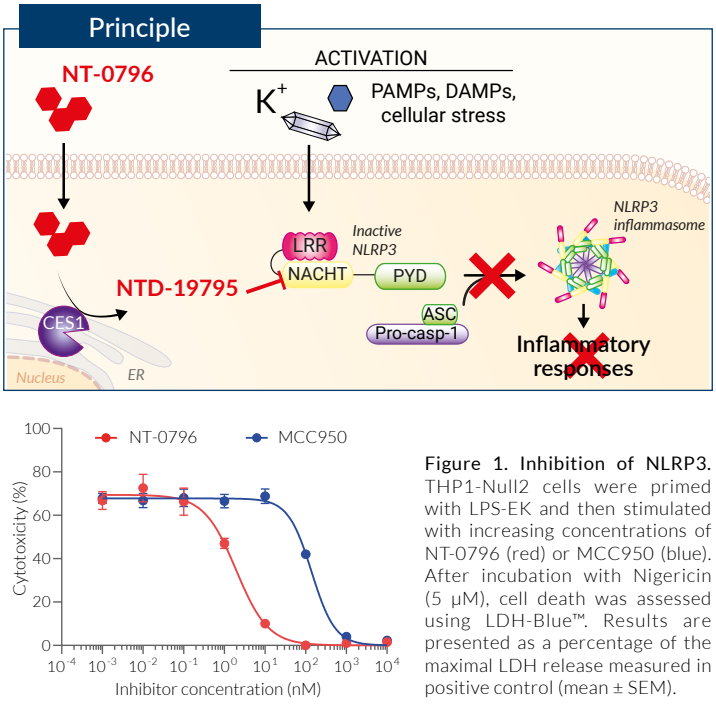


Figure 1. Inhibition of NLRP3. THP1-Null2 cells were primed with LPS-EK and then stimulated with increasing concentrations of NT-0796 (red) or MCC950 (blue). After incubation with Nigericin (5 µM), cell death was assessed using LDH-Blue™. Results are presented as a percentage of the maximal LDH release measured in positive control (mean ± SEM).

Learn more here www.invivogen.com/inflammasome-inhibitors

1. Harrison D, *et al.*, 2023. J Med Chem. 9;66(21):14897-14911.

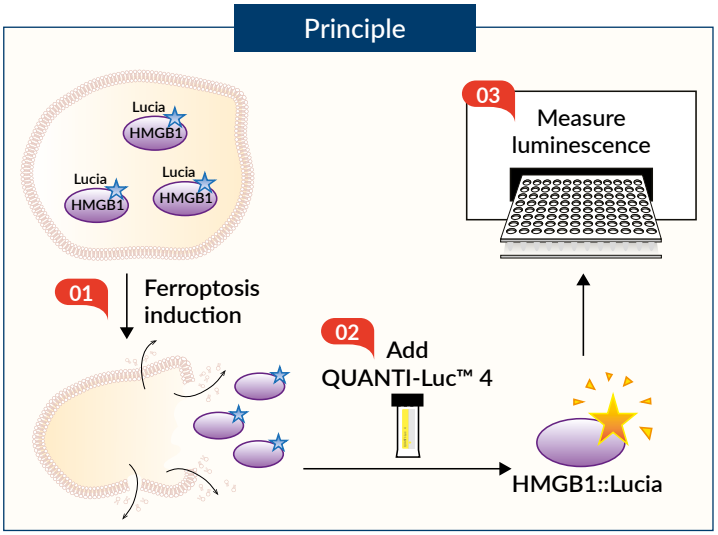
Ferroptosis bioassay

Ferroptosis, a disctinct form of regulated cell death driven by iron-dependent lipid peroxidation, plays a critical role in cancer progression and neurodegenerative diseases. To support ferroptosis research, InvivoGen has developed a robust and sensitive bioassay based on HMGB1-Lucia™ reporter cells, derived from the human fibrosarcoma cell line HT-1080, the gold-standard used in ferroptosis studies. Our Ferroptosis bioassay allows precise monitoring of ferroptotic cell death induction or inhibition and screen for potential therapeutics.

- **HT1080-HMGB1-Lucia™ reporter cells** NEW
- **Ferroptosis inducer RSL3** NEW
- **Ferroptosis inhibitor Ferrostatin-1** NEW

Ferroptosis is characterized by the build-up of lipid reactive oxygen species (ROS) triggering membrane damage which can be monitored through the release of HMGB1, a nuclear 'alarmin' that serves as a cell death-associated damage-associated molecular pattern.

InvivoGen's ferroptosis bioassay enables researchers to study the regulation of this process, offering a reliable system to screen for novel ferroptosis inducers and inhibitors in order to uncover new therapeutic targets.



Key Features

- Real-time luminescent detection of HMGB1::Lucia release
- Reliable assay tools for screening and mechanistic studies
- Lot-to-lot tested and validated

The **HT1080-HMGB1-Lucia™ Reporter Cells** provide a highly sensitive, luminescent-based system for detecting HMGB1::Lucia release in response to ferroptotic stimuli. These cells enable precise quantification of ferroptotic cell death, using the QUANTI-Luc™ 4 Lucia/Gaussia detection reagent *(Fig.1)*. Additionally, ferroptosis in this cell line can be assessed using the classic cytotoxic lactate dehydrogenase assay *(Fig.2)*.

To actively induce ferroptosis, **RSL3** (RAS-selective lethal 3) directly inhibits glutathione peroxidase 4 (GPX4), an essential enzyme responsible for neutralizing lipid peroxides. GPX4 inhibition by RSL3 leads to an uncontrolled accumulation of lipid peroxidation, resulting in iron-dependent cell death *(Fig.1A)*.

Conversely, **Ferrostatin-1** acts as a potent ferroptosis-specific inhibitor by scavenging lipid peroxyl radicals and preventing cell death *(Fig.3)*. Its ability to rescue cells from ferroptosis makes it a valuable tool for studying the protective mechanisms against oxidative damage and the potential development of ferroptosis-targeted therapies.

PRODUCTS	QTY	CAT. CODE
HT1080-HMGB1-Lucia™ Cells	2 - 5 x 10^6	ht80-gb1lc
RSL3	10 mg	inh-rsl3
Ferrostatin-1	10 mg	inh-fers1

RELATED PRODUCTS

- **QUANTI-Luc™ 4:** Luciferase detection reagent, liquid (rep-qlc4lg1)
- **LDH-Blue™:** LDH activity assay kit (rep-ldh-1)

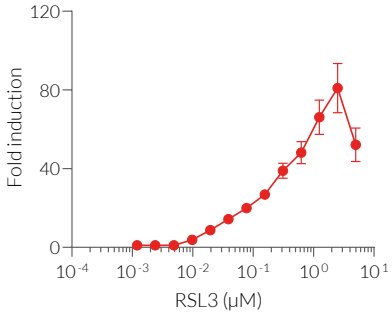


Figure 1. RSL3 induces release of HMGB1::Lucia in a dose-dependent manner. HT1080-HMGB1-Lucia™ cells were incubated with increasing concentrations of RSL3. After 48h, the induction of cell death was quantified by measuring HMGB1::Lucia in the supernatant using QUANTI-Luc™. Data are shown as fold induction over non-induced cells (mean ± SEM).

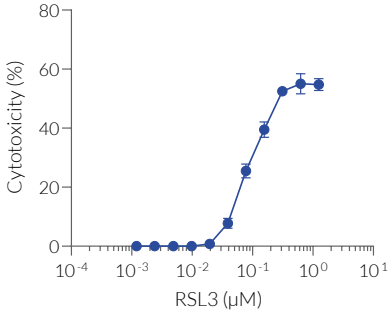


Figure 2. RSL3 induces release of LDH in a dose-dependent manner. Cells were treated as described above. After 48h, ferroptosis induction was quantified using the lactate dehydrogenase (LDH) assay LDH-Blue™. Results are presented as a percentage of the maximal LDH release measured in positive control (mean ± SEM).

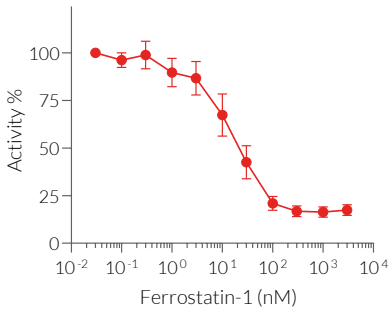


Figure 3. Ferrostatin-1 inhibits RSL3-induced HMGB1::Lucia release in a dose-dependent manner. Cells were incubated with increasing concentrations of Ferrostatin-1 for 30 min followed by the addition of the ferroptosis inducer RSL3 (1 µM). After 48h, the inhibition of cell death was quantified by measuring HMGB1::Lucia in the supernatant using the QUANTI-Luc™. Data are shown as percentage (%) activity (mean ± SEM).

Learn more here www.invivogen.com/regulated-cell-death

Cytotoxic assay kit

LDH-Blue™ is a new colorimetric assay for measuring lactate dehydrogenase (LDH) released by damaged cells in the medium. This innovative kit is based on an enzymatic reaction that leads to the formation of blue formazan, changing the color of the medium from pale red to purple. The color change, which is visible with the naked eye, is quantifiable and directly proportional to cell viability. LDH-Blue™ gives more robust and precise readout of cell death than classical LDH assays that use red formazan.

- LDH-Blue™ **NEW**

Key Features

- Pratical format: Add, Mix & Read
- Visual results in < 30 min
- No interference with phenol red
- Reliable performance, cost-efficient excellence

LDH is a ubiquitous cytosolic enzyme that is released into the extracellular environment upon plasma membrane damage. Its stability in cell culture medium makes it a well-suited marker for cellular toxicity. LDH-Blue™ provides a robust readout of lytic cell death, including necroptosis, pyroptosis, ferroptosis, late apoptosis, and immune cell-mediated cytotoxicity *(Fig. 1 & 2)*.

LDH-Blue™ is a four-component kit that comprises a Reagent Mix, an Assay Buffer, a Lysis Buffer, and a Stop Solution. The Reagent Mix is a lyophilized mixture containing the substrate which - upon reduction - produces blue formazan. This reaction changes the color of the medium from pale red to purple, is visible by the naked eye, and quantifiable at 650 nm. The use of blue formazan induces no interference with phenol red in the medium leading to more accurate readout of cell death than classical LDH assays that use red formazan.

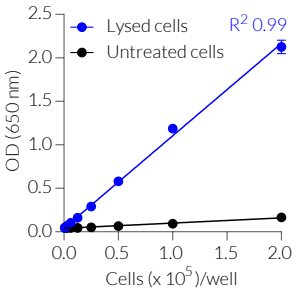


Figure 1. Linear relationship between the number of lysed cells and the activity of released LDH. Increasing concentrations of THP1-Null2 cells were cultured for 30 min either with (blue) or without (black) Lysis Buffer. After incubation, the LDH release was quantified in the supernatant as per the instructions in the LDH-Blue™ technical data sheet. Data is shown as optical density (OD) at 650 nm (mean ± SEM).

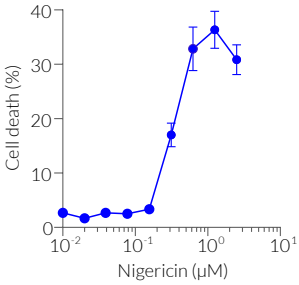
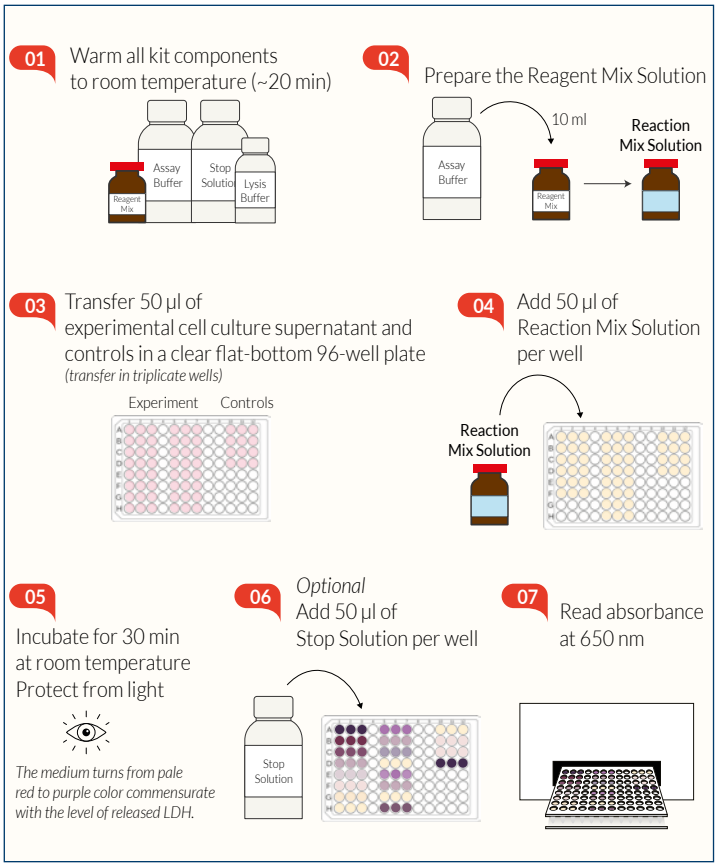


Figure 2. Pyroptotic cell death detection using LDH-Blue™. THP1-Null2 cells were primed with LPS-EK and then incubated with increasing concentrations of Nigericin, an NLRP3 inflammasome inducer. Results are presented as a percentage of the maximal LDH release measured in positive control (mean ± SEM).

PRODUCT	QTY	CAT. CODE
LDH-Blue™	200 / 5 x 200 / 50 x 200 tests*	rep-ldh-1/ rep-ldh-5/ rep-ldh-50

** 200 tests = 2 x 96-well plates*

LDH-Blue™ assay at a glance



Learn more here
www.invivogen.com/cell-viability-assay-cell-death-assay-cytotoxicity-ldh-blue-kit

InvivoGen

Europe Tel: +33 562 71 69 39 Fax: +33 562 71 69 30 info.eu@invivogen.com
USA Tel: +1 888 457 5873 Fax: +1 858 457 5843 info@invivogen.com
Asia Tel: +852 3622 3480 Fax: +852 3622 3483 info.hk@invivogen.com