HT1080-HMGB1-Lucia™ Cells

Ferroptosis reporter HT-1080 cells

Catalog code: ht80-gb1lc

https://www.invivogen.com/ht1080-ferroptosis-reporter-cells

For research use only

Version 25C03-AK

PRODUCT INFORMATION

Content

- **2-5 x 10^6 of HT1080-HMGB1-Lucia™ cells in a cryovial or shipping flask.** *IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.*
- **1 ml of Blasticidin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), a formulation of 3 antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

- **1 tube of QUANTI-Luc™ 4 Reagent**, a lucia luciferase detection reagent (sufficient to prepare 25 ml). Store at -20 °C. Avoid repeated freeze-thaw cycles. *Note: This product is photosensitive and should be protected from light.*

Note: Data sheets for all components are available on our website.

Handling Frozen Cells Upon Arrival

Cells are shipped in dry ice, and upon receipt should immediately be thawed for culture or stored below -130°C, preferably in liquid nitrogen vapor, for long-term storage.

IMPORTANT: Do not store cell vials at -80°C as this will decrease cell viability and performance. Contact technical support if the cells are not frozen or in dry ice upon arrival.

To insure the highest level of viability and best assay performance, we strongly recommend that you thaw the cells and initiate the culture as soon as possible upon receipt (as described on the next page).

Warranties

- InvivoGen's cells are provided 'AS IS' and their viability is guaranteed upon shipment from our facilities for a period of 30 days, provided that the customer has properly stored and handled the product.
- Our cell lines are guaranteed free of mycoplasma contamination.
- The stability of our cell lines is guaranteed for 20 passages.

Quality Control

- The release of the HMGB1::Lucia reporter protein upon ferroptosis induction has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- The cell line is guaranteed mycoplasma-free.

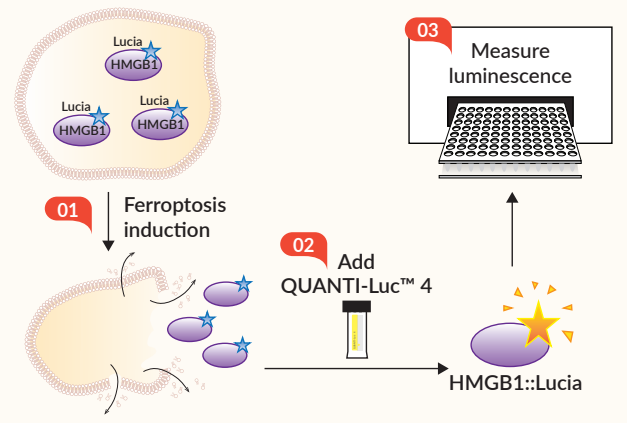
PRODUCT DESCRIPTION

HT1080-HMGB1-Lucia™ cells are derived from the human fibrosarcoma HT-1080, a standard model to study ferroptosis.

HT1080-HMGB1-Lucia™ cells stably express a 46.5 kDa fusion protein, HMGB1::Lucia, in the cytoplasm in which the C-terminus of the High-Mobility-Group-Protein B1 (HMGB1) is fused to the Lucia® luciferase. Following ferroptosis activation, the cell membrane ruptures and HMGB1::Lucia is released 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™ 4 Lucia/Gaussia, a Lucia® luciferase detection reagent.

This assay is an alternative to the lactate dehydrogenase (LDH) assay which measures the activity of LDH released upon rupture of cell membrane integrity.

HT1080-HMGB1-Lucia™ cells are selectable with Blasticidin.



HMGB1::Lucia protein release upon ferroptosis induction

USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact outlicensing@invivogen.com.

TECHNICAL SUPPORT
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FAQ Any questions about our cell lines? Visit our FAQ page.

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS; 30 min at 56°C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 20% (v/v) FBS, 10% (v/v) DMSO
- **Test Medium:** DMEM 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Blasticidin or Normocin™.**

Required Selective Antibiotics

Blasticidin

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% (v/v) ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells into a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 300 x g (RCF) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the flask containing cells at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 1-4 x 10^6 cells/ml in freshly prepared freezing medium with cold DMEM.
2. Aliquot 1 ml cells into cryogenic vials.
3. Place vials in a freezing container and store at -80 °C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. HT1080-HMGB1-Lucia™ cells grow as adherent cells. Detach the cells using **undiluted** trypsin for 2-3 min at room temperature (RT).

Note: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of receptors.

2. After cells have recovered (after at least one passage), subculture the cells in growth medium supplemented with 10 µg/ml of Blasticidin.
3. Renew growth medium twice a week.
4. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: The doubling time of this cell line is ~50 hours.

REPORTER ASSAY PROTOCOL

Below is a protocol using HT1080-HMGB1-Lucia™ cells to monitor ferroptosis. It is recommended to perform the assay with test medium which does not contain Normocin™ nor Blasticidin.

Cell preparation

Day 1

1. Split confluent cells to 1:2

Day 2

1. Remove old media and wash cells with 1X PBS.
2. Detach cells using 1 ml of **undiluted** trypsin (for a T75) and incubate for 2-3 minutes at RT.
3. Add 9 ml of test media and count cells.
4. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~50,000 cells/ml.
5. Add 200 µl of the cell suspension per well (20,000 cells/well).
6. To enable cell adhesion, incubate at 37°C in 5% CO_2 for 2 h.
7. Proceed with ferroptosis induction assay.

Ferroptosis induction assay

1. Remove old media and add 180 µl of fresh test media.
2. Add 20 µl of RSL3 per well (1 µM final concentration). Include negative control (e.g. endotoxin free water).
3. Incubate at 37°C in 5% CO_2 for 48 h.

Day 4

This cellular assay relies on the luminescence quantification of the HMGB1::Lucia fusion protein released in the supernatant upon the ferroptotic cell death, by using QUANTI-Luc™ 4 Reagent.

Detection of the released HMGB1::Lucia protein

Below is a protocol for end-point readings using a luminometer with an injector. This protocol can be adapted for use with a luminometer with or without an injector for kinetic measurements.

1. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet (see attached).
2. Transfer 20 µl of cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ 4 Reagent
4. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. Code
RSL3	inh-rsl3
Ferrostatin-1	inh-fers1
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1
Blasticidin	ant-bl-05
Normocin™	ant-nr-1

TECHNICAL SUPPORT
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QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

https://www.invivogen.com/quanti-luc

For research use only

Version 24G30-MM

PRODUCT INFORMATION

Contents

- 1 tube of **QUANTI-Luc™ 4 Reagent (20X)**

  One tube of QUANTI-Luc™ 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia or Renilla kits.

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

– Store QUANTI-Luc™ 4 Reagent at -20°C for up to 12 months.
– After preparation, the working solution is stable for 48 hours at 4°C and 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.
Note: This product is photosensitive and should be protected from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
– Physicochemical characterization (pH, appearance).
– Functional assays using recombinant Lucia® protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is one component of the QUANTI-Luc™ 4 Lucia/Gaussia and QUANTI-Luc™ 4 Renilla kits. It contains the coelenterazine substrate for the detection of secreted Lucia® or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

Note: Lucia® is a registered trademark of InvivoGen.

METHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
2. Vortex **very briefly** (a few seconds).
3. Use the working solution immediately or store until required for use. QUANTI-Luc™ 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash detection of Lucia® luciferase activity in cell culture medium:

To obtain **end-point readings** using a luminometer **with an injector**.
1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with QUANTI-Luc™ 4 Reagent 1X and proceed **immediately** with the measurement.

To obtain **end-point readings** using a luminometer **without injectors**.
1. Set the luminometer with a 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ 4 Reagent 1X to each well or tube.
4. Gently tap the plate several times to mix (do **not** vortex).
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia Kit comprising QUANTI-Luc™ 4 Reagent & Stabilizer	rep-qlc4lg1
QUANTI-Luc™ 4 Renilla Kit comprising QUANTI-Luc™ 4 Reagent & Lysis buffer	rep-qlc4r1

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