Product Specification Sheet

Product Name	**Nanoluciferase/tdTomato Dual-Reporter HCT116 Cell Line**
Description	NanoLuc luciferase is a small (19 kDa), highly stable, ATP independent, bioluminescent protein. Compared to firefly luciferase, it is an extremely robust and ultra high sensitivity screening system. It has been widely used as convenient and powerful reporting tools in numerous biomedical researches, such as, cell tagging, protein-protein interaction assays, real-time monitoring and high-throughput drug screening. Nanoluciferase/tdTomato dual-reporter HCT116 (HCT116-NanoLuc-RFP) cell line is derived from HCT116 cells by transduction of ALSTEM's pLenti-CAG-Nanoluciferase-PGK-tdTomator-T2A-PURO Lentiviral Reporter (cat# LV311L) lentivirus, which contains Firefly Nanoluciferase encoding gene driven by CAG promoter, followed by tdTomato and puromycin resistance gene by PGK promoter. This cell line stably expresses Nanoluciferase at high level. It also expresses a red fluorescent protein (tdTomato) and is resistant to puromycin. This cell line will be a very useful cell line for non-invasive visualization in both in vitro and in vivo experiments.
Catalog Number	NRL02
Size	1 x10^6 cells/vial
Shipping	Dry ice
Storage and Stability	Store in vapor phase of liquid nitrogen immediately upon receipt. This product is stable for 6 months when stored as directed.
Quality Control	Each vial contains about 1 x 10^6 cells with >95% viability before freezing. Each lot of cells is tested for Nanoluciferase activity, growth and viability following recovery from cryopreservation, and free of mycoplasma and competent lentivirus as well.
Safety Precaution	**ALSTEM highly recommends that protective gloves, a lab coat, and a full-face mask are always worn when handling frozen vials.** It is important to note that some liquid nitrogen can leak into the vials when submersed in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in excessive pressure within the vial that can cause the vial to explode or expel the cap with dangerous force.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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Overview

NanoLuc luciferase is a small (19 kDa), highly stable, ATP independent, bioluminescent protein. Compared to firefly luciferase, it is an extremely robust and ultra high sensitivity screening system. It has been widely used as convenient and powerful reporting tools in numerous biomedical researches, such as, cell tagging, protein-protein interaction assays, real-time monitoring and high-throughput drug screening.
Nanoluciferase/tdTomato dual-reporter HCT116 (HCT116-NanoLuc-RFP) cell line is derived from HCT116 cells by transduction of ALSTEM's pLenti-CAG-Nanoluciferase-PGK-tdTomator-T2A-PURO Lentiviral Reporter (cat# LV311L) lentivirus, which contains Firefly Nanoluciferase encoding gene driven by CAG promoter, followed by tdTomato and puromycin resistance gene by PGK promoter. This cell line stably expresses Nanoluciferase at high level. It also expresses a red fluorescent protein (tdTomato) and is resistant to puromycin. This cell line will be a very useful cell line for non-invasive visualization in both in vitro and in vivo experiments.

Characterization:

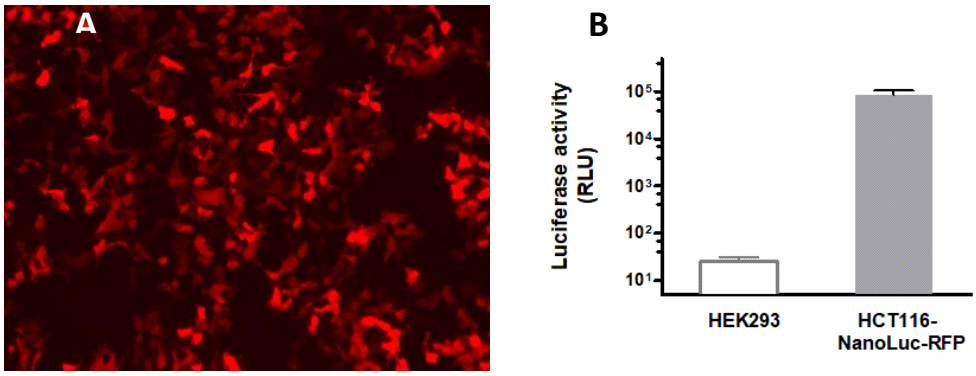

Figure 1. Panel A: RFP Fluorescence. Dual-labeled HCT116 cell line expressing tdTomato at 80% cell confluence. Panel B: Nanoluciferase activity. Comparison of luminescence signal for NanoLuc luciferase and negative control demonstrating its robustness and high sensitivity in the Nanoluciferase/tdTomato dual-labeled HCT116 cells. The Nanoluciferase activity was tested 6 hrs after seeding in a 96-well plate. Data are expressed as mean +/- SD from duplicates of three independent experiments.

Vector Information

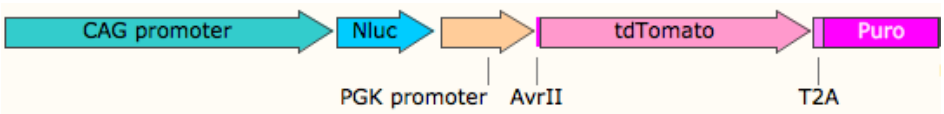

Figure 2. The lentiviral reporter vector contains Nanoluciferase gene driven by CAG promoter, tdTomato and puromycin resistance gene by PGK promoter.

Protocol

Culture Medium:
90% DMEM with GlutaMAX™-I (high glucose) (Thermo Fisher, Cat# 10569-010),
+ 10% Fetal bovine serum (FBS) (Gibco, Cat# 26400-036)
+ 1% Penn/Strep (Thermo Fisher, Cat# 15140-122)

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Thawing cells

1. Add 5 mL of fresh culture medium into a T25 flask.
2. Place the flask in a humidified 37 °C / 5% CO_2 incubator for 15 min to allow medium to equilibrate to the proper pH and temperature.
3. Take the vial of cells from liquid nitrogen and rapidly thaw by placing at 37 °C in a water bath with gentle agitation until half thawed. (About 1–2 min, do not submerge vial in water.)
4. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
5. Transfer the vial contents to a sterile 15-mL conical tube. Add 10 mL of warm culture medium drop-wise into the tube, gently mixing as the medium is added.
6. Centrifuge cells at 200 × g for 5 min at room temperature.
7. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh culture medium.
8. Transfer contents to the T-25 flask containing pre-equilibrated culture medium and place flask in a humidified 37 °C / 5% CO_2 incubator.
9. Change culture medium every other day or when pH decreases. Monitor the cells every day.

Maintenance of cells

1. Cells were split when they reach about 85% confluence.
2. Pre-warm fresh culture medium in fresh culture vessel in the incubator.
3. Aspirate medium from growing cells, rinse once with PBS, add 0.05% Trypsin/EDTA (2 mL for a T-25 flask, 5 mL for a T-75 flask, 10 mL for a T-175 flask) and swirl to evenly cover the cells. Incubate for 3min. After 3 min, tap the flask firmly on the side. Cells usually detach easily.
4. Add an equal volume of culture medium (10 mL for a T-175 flask) to inactivate Trypsin/EDTA, and break up the cell clumps by gently pipetting up and down several times.
5. Verify under a microscope that cells have detached and clumps have completely dispersed.
6. Count the cell number using a hemocytometer. Dilute cells 1:1 with 0.4% trypan blue. Add 10ul to a hemocytometer and count the cells. Count at least 4 quandrants.
7. Centrifuge cells at 200 × g for 5 min at room temperature.
8. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh culture medium.
9. Plate $2x10^6$ cells per T-175 flask.
10. Media volume for each T-175 flask would be 20ml.
11. Incubate at a humidified 37 °C / 5% CO_2 incubator.
12. Change media every other day, until the cells reach 85% confluence. Monitor the cells every day. The cells should be 60-70% confluent, three days after plating.

Freezing cells

1. Aspirate the medium and wash the cells twice with 20 ml of warm DPBS per T-175 flask.
2. Aspirate DPBS, and add 10 ml of 0.05% trypsin-EDTA per T-175 flask, and incubate for 2-3 min. After 3 min, tap the flask firmly on the side. The cells should be observed coming off the flask easily.
3. Add 10 ml of culture medium and break up the cell clumps by gently pipetting up and down several times.

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4. Transfer the cells to a 50 mL conical tube per T-175 flask. Count the number of cells using 0.4% trypan blue.
5. Centrifuge at 200 g for 5 min at room temperature.
6. Aspirate the supernatant, resuspend the pellet in cold EZStem™ Freezing Media (Cat# M050) at $1x10^6$ cells/ml.
7. Transfer 1 mL of cell suspension in freezing medium into each labeled cryogenic vial.
8. Place the vials in a Mr. Frosty cell-freezing container and keep it at -80 °C overnight.
9. Transfer the vials to a liquid nitrogen tank for long-term storage.

Nanoluciferase assay

Nanoluciferase assay was performed according to the instruction of Nano-Glo® Luciferase Assay System from Promega

Nano-Glo® Luciferase Assay System yields reliable and robust results in high-throughput screening applications. The Nano-Glo® Reagent provides stable luminescence with a half-life of approximately 120 minutes at room temperature.

1. Preparing the fresh Nano-Glo® Luciferase Assay Reagent according to manufacturer's instruction before experiment.
2. Remove 96-plate containing the cells from the incubator. The plate should fit into the luminometer. For best results, equilibrate the plate to room temperature before performing Step 3.
3. To each well, add a volume of Nano-Glo® Reagent equal to the volume of culture medium in the well, and mix. (To each well of a 96-well plate, typically 100µl of freshly prepared reagent is added, which is equal to the volume of cell culture medium.)
4. Measure luminescence after waiting at least 3 minutes. The luminescence intensity will decay gradually, with a signal half-life of approximately 120 minutes at room temperature.

IMPORTANT NOTICE

Store the vials at vapor phase of liquid nitrogen immediately upon receipt.

WARNING

Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquid nitrogen inside the vial and lead to pressure buildup resulting in possible explosion or biohazard release. Use appropriate safety procedures which are outlined by the ATCC when handling and disposing of vials. ALSTEM highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials.