Product Specification Sheet

Product Name	**Luciferase/tdTomato Dual-Reporter Jurkat Cell Line**
Description	Luciferase catalyzes the substrate luciferin in the presence of Mg2+ and ATP, resulting in a flash of light that decays rapidly. This light can be detected by a luminometer. Luciferase from the firefly has become one of the more widely used reporter proteins for both in vitro and in vivo studies, such as, gene transcription activity assay, the noninvasive imaging of reporter gene expression, imaging cerebral strokes, and tracking genetically engineered T cells. Luciferase/tdTomato dual-reporter Jurkat (Jurkat -Luc-RFP) cell line is derived from Jurkat cells by transduction of ALSTEM's pLenti-SFFV-Luciferase-PGK-tdTomator-T2A-PURO Lentiviral Reporter (cat# LV452) lentivirus, which contains Firefly luciferase encoding gene driven by SFFV promoter, followed by tdTomato and puromycin resistance gene by PGK promoter. This cell line stably expresses luciferase 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	LRL06
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 luciferase 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

Luciferase catalyzes the substrate luciferin in the presence of Mg2+ and ATP, resulting in a flash of light that decays rapidly. This light can be detected by a luminometer. Luciferase from the firefly has become one of the more widely used reporter proteins for both in vitro and in vivo studies, such as, gene transcription activity assay, the noninvasive imaging of reporter gene expression, imaging cerebral strokes, and tracking genetically engineered T cells. Luciferase/tdTomato dual-reporter Jurkat (Jurkat -Luc-RFP) cell line is derived from Jurkat cells by transduction of ALSTEM's pLenti-SFFV-Luciferase-PGK-tdTomator-T2A-PURO Lentiviral Reporter (cat# LV452) lentivirus, which contains Firefly luciferase encoding gene driven by SFFV promoter, followed by tdTomato and puromycin resistance gene by PGK promoter. This cell line stably expresses luciferase 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:

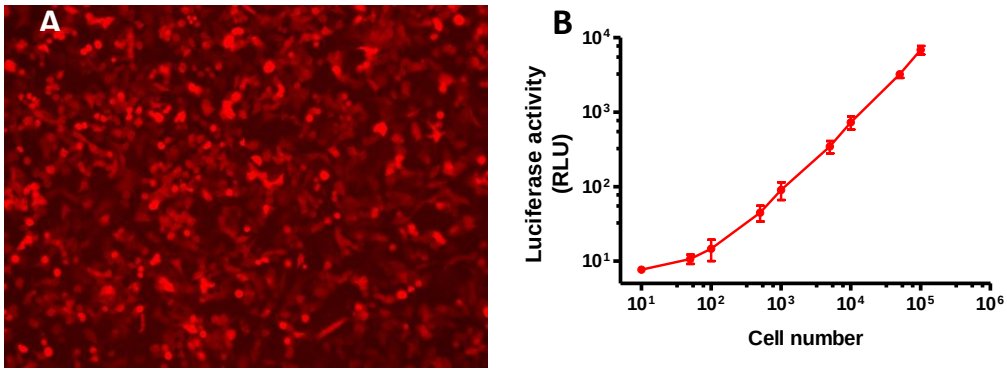

Figure 1. Panel A: RFP Fluorescence. Dual-labeled Jurkat cell line expressing tdTomato at 90% cell confluence. The image was taken using a Nikon fluorescent microscope. Panel B: Luciferase activity. Serial dilutions of dual-labeled Jurkat cells were plated into a 96-well plate. The luciferase activity was tested 6 hrs later. Data are expressed as mean +/- SD from duplicates of three independent experiments.

Vector Information

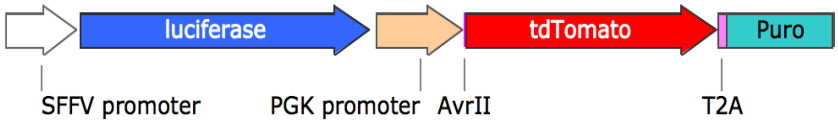

Figure 2. The lentiviral reporter vector contains luciferase gene driven by SFFV promoter, tdTomato and puromycin resistance gene by PGK promoter.

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.

Luciferase assay

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

Steady-Glo® Luciferase Assay System yields reliable and robust results in high-throughput screening applications. The Steady-Glo® Reagent provides stable luminescence with a half-life of greater than 5 hours when used with common cell culture media.

1. Remove 96- plates containing mammalian cells from the incubator. The plates used must be compatible with the luminometer being used. For best results, equilibrate cultured cells to room temperature before performing Step 2.
2. To each plate well, add a volume of Steady-Glo® Reagent equal to the volume of culture medium in the well, and mix. (For 96-well plates, typically 100µl of reagent is added to cells grown in 100µl of medium.)
3. Wait at least 5 minutes to allow cell lysis, then measure luminescence in a luminometer.

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.