Applied StemCell, Inc.
(866) 497-4180
www.appliedstemcell.com

Datasheet

Human Induced Pluripotent Stem Cell TARGATT™ GFP Reporter Line

Product Information

Catalog Number	**ASE-9505**
Description	Applied StemCell, Inc. provides a control Human Induced Pluripotent Stem Cell (hiPSC) TARGATT™ GFP Reporter Line at a low passage (p7). These TARGATT™ GFP pluripotent cells were generated from the ASE-9211 master iPSC line. An integrase recognition landing pad was engineered into the ASE-9211 iPSC line for site-specific integration. The engineered ASE-9211 parental line was transfected with the TARGATT™ CAG-GFP-TK positive control plasmid and unique integrase plasmid. Integrase expression mediated the stable integration of GFP at the landing pad. The resulting human iPSC TARGATT™ GFP cells have been tested for morphology (Figure 1), the expression of pluripotency markers (Oct4, Sox2, and Nanog; Figure 2), and GFP expression (Figure 3). Detailed protocols for thawing, culturing under feeder-free conditions, and cryopreservation of these iPS cells are provided.
Parental Line	ASE-9211; Human Induced Pluripotent Stem Cells (from Normal, Male, Human Fibroblasts)
Passage #	P7
Quantity	1 x 10^6 cells/vial
Shipping	Dry ice
Storage and Stability	Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.
Quality Control	Each lot of human iPS cells has been tested for growth and viability following recovery from cryopreservation, morphology, immunohistochemistry for pluripotency markers: *Oct4, Sox2, Nanog*; karyotyping; and for the absence of bacteria, fungi, mycoplasma (CoA available upon request).
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling frozen vials. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.
Restricted Use	This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Applied StemCell, Inc.
521 Cottonwood Dr. #111, Milpitas, CA 95035
Phone: 866-497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com

Characterization of iPSC Line ASE-9505

Morphology Images

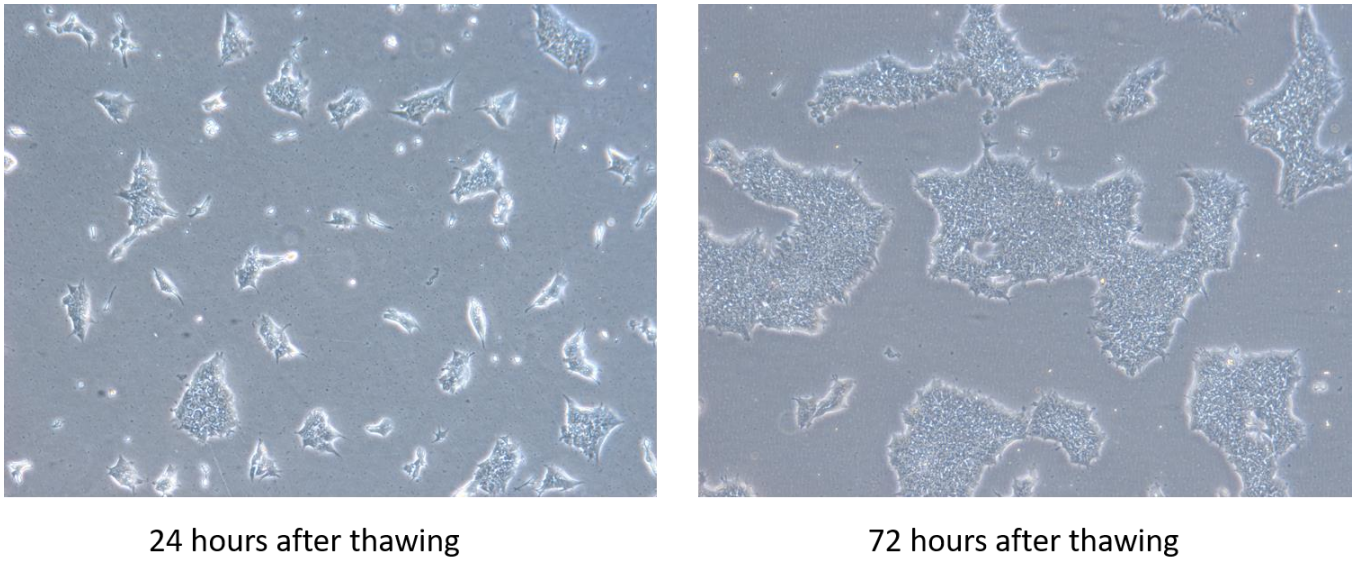

Figure 1: Morphology images of the ASE-9505 iPSC TARGATT™ GFP Reporter Line captured 24 hours and 72 hours post thaw. The ASE-9505 presented good morphology 24 and 72 hours post thaw. The image on the left was taken 24 hours after thawing and the image on the right was captured 72 hours after thawing.

Pluripotency Marker Analysis

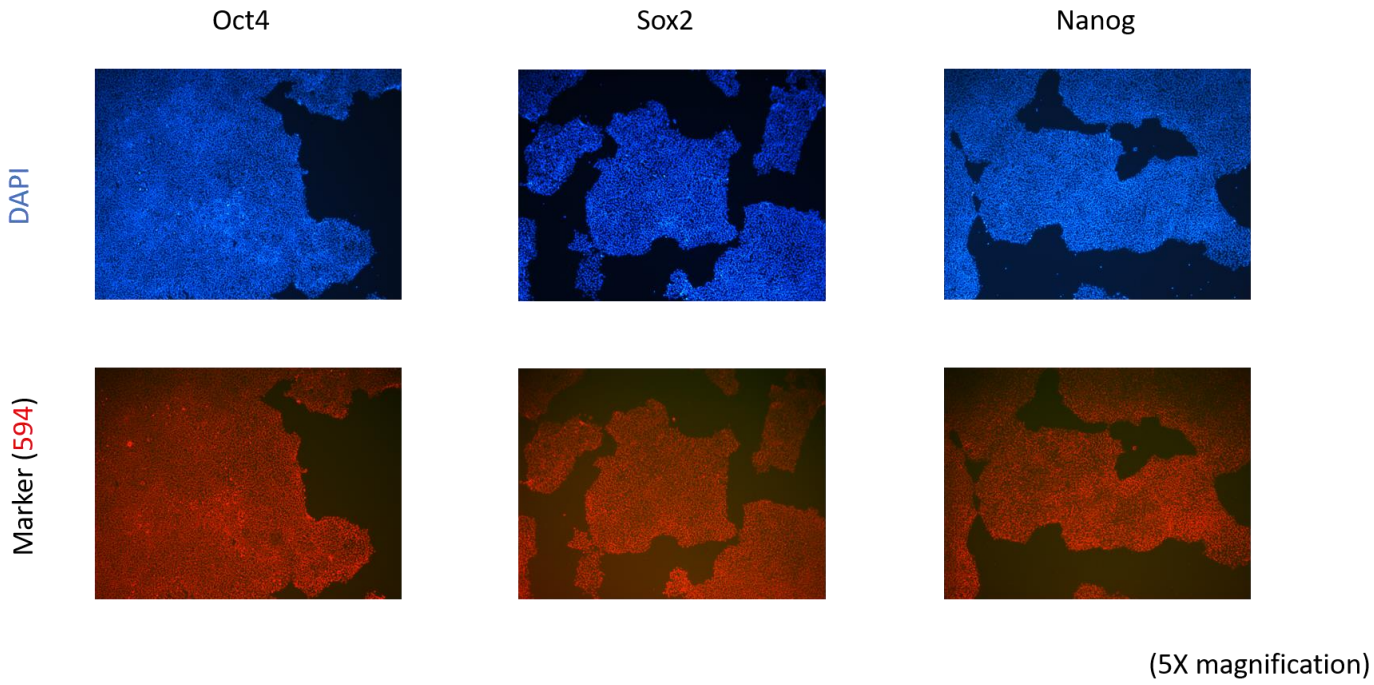

Figure 2: Expression of pluripotency markers. The ASE-9505 iPSC line expresses common iPSC biomarkers (bottom row: Oct4, Sox2, and Nanog). Top row: Corresponding DAPI nuclear staining. All images were taken at 5X magnification.

GFP Expression Analysis

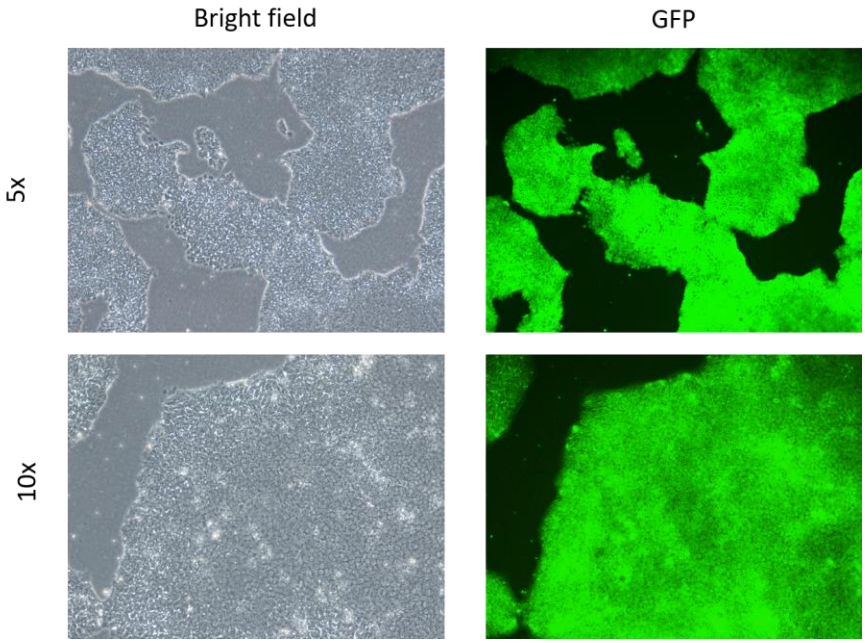

Figure 3: GFP expresses 5 days post thawing. The TARGATT™ CAG-GFP-TK plasmid was used to generate the ASE-9505 iPSC TARGATT™ GFP Reporter Line. A GFP signal was detected by fluorescence imaging 5 days post thawing. (Left Images: Bright field microcopy; Right Images: Immunofluorescence; Top Row: 5x magnification; Bottom Row: 10x magnification)

Media and Material Required but not Provided

- mTeSR-Plus Medium, StemCell Technologies, Cat# 05825
- Matrigel®, Corning, Cat# 354277
- Geltrex™ LDEV-Free Reduce Growth Factor Basement Membrane Matrix, ThermoFisher Scientific, Cat# A1413202 (Matrigel®, Corning Matrix Alternative)
- ROCK Inhibitor (Y-27632), Sigma-Aldrich, Cat# SCM075
- CryoStor® CS10, StemCell Technologies, Cat# 7930
- 0.5M EDTA in PBS, ThermoFisher Scientific, Cat# 15575-020
- PBS, Life Technologies, Cat# 14190136
- 100 µg/mL Primocin™, Invivogen, Cat# ant-pm-2 or 1% Penicillin- Streptomycin, Life Technologies, Cat# 15140
- Cell Scraper, VWR International, Cat# 75799-938
- Corning® CoolCell® FTS30, Corning®, Cat# 432006

Protocol

Feeder-free culture conditions

1. Thawing human iPSCs using a feeder-free protocol

1.1 Prepare Matrigel® or GelTrex™ coated 6-well plates in advance, following vendor's instructions.
1.2 Prepare 6 mL of mTeSR-plus medium + 10 µM Rock Inhibitor.
1.3 Add 1 mL of mTeSR-plus medium + Rock Inhibitor in one well of a Matrigel®-coated (or GelTrex™ plate). Prepare 3 wells for each vial of frozen cells.
1.4 Bring the cryovial on dry ice to the tissue culture room.
1.5 Quickly thaw the iPSCs in a 37°C water bath, by gently shaking the cryovial continuously until only a small piece of ice remains.
1.6 Wipe the cryovial with a paper towel sprayed with 70% ethanol and place it into a biosafety cabinet.
1.7 Add 9 mL of mTeSR-plus medium to a 15 mL conical tube.
1.8 Using a 1 mL pipette transfer the cells to the 15 mL conical tube dropwise while swirling the conical tube.
1.9 Centrifuge the cells for 3 min at 200 RCF and at room temperature.
1.10 Aspirate off the medium, and add 4 mL mTeSR-plus medium + Rock Inhibitor.
1.11 Gently flick the conical tube to resuspend the cells and transfer them to the 2 wells of the Matrigel® plate (or GelTrex™ plate) using a 5 mL serological pipette.
*Note: Prepare Matrigel plates (or GelTrex™ plate) the day before and not more than 3 days prior to thawing. Before transferring the cells, the Matrigel (or GelTrex™) **must** be aspirated.*

1.12 Place the plate in the incubator and move the plate back-and-forth and side-to-side, twice to spread the clumps evenly in the wells.
1.13 Medium can be changed 2 days after thawing. After the 2 days, if the number of attached colonies is still low (less than 5% confluency), change half of the medium (aspirate 1 mL and add 1 mL of fresh medium).
1.14 Once colonies are stabilized, change the medium daily. Usually, within 1 week the cells are ready to be split.
1.15 When the ASE-9505 hiPSC colonies are big or close enough to merge, the cells need splitting/ passaging.

2. Passaging/ splitting human iPSCs using EDTA

2.1 Aspirate the medium from the hiPSC culture.
2.2 Wash once with 1 mL of 0.5 mM EDTA (in PBS).
2.3 Aspirate the EDTA and add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3 min in a 37°C incubator.
2.4 Observe the cells under a microscope until the cells at the edge of the colonies start to separate and round up.
2.5 With the cells still attached, aspirate the EDTA and add 1 mL of mTeSR-plus medium.
2.6 Scrape the cells from the bottom of the well until the colonies are floating; pipette up and down 2-3 times (with the 1000 µL pipette set to 800 µL) to break the colonies in small clumps.
Note: Pipetting up and down three times is enough to break the colonies into clumps of optimal size.

2.7 Transfer the desired dilution to the wells of the new Matrigel®-coated plate (or GelTrex™ plate) (usually around a 1:3 splitting ratio).
Note: Prepare Matrigel plates (or any other basement matrix) not more than 3 days prior to passaging cells

2.8 Place the plate in the incubator and move the plate back and forth and side to side, twice to spread the clumps evenly in all the wells.
Note: hiPSCs are passaged as clumps of 50-200 cells, rather than single cells. Cells need to be passaged before the colonies are large enough to merge with one another.

3. Cryopreserving human iPS cells

3.1 Label the cryovial as needed, based on 1 vial per well of a 6-well plate, and pre-chill them in a 4°C freezer.

3.2 Aspirate the medium from the hiPSC culture.

3.3 Wash once with 1 mL of PBS.

3.4 Aspirate the PBS and add 1 mL of 0.5 mM EDTA (in PBS) per well of a 6-well plate and incubate the cells for 3-5 minutes in a 37°C incubator.

3.5 Observe the cells under a microscope. After 1-2 minutes the cells at the edge of the colonies will start to separate and round up.

3.6 Once round colonies are observed, aspirate the EDTA (even if it hasn't been 5 minutes of incubation).

3.7 Add 1 mL of cold freezing medium, CS10 and scrape the cells from the bottom of the well until the colonies are floating.
Note: The freezing medium must remain at 4°C until usage.

3.8 Pipette the cells once or twice to break up any big clumps before transferring the suspension to a cryovial.

3.9 Transfer the cell suspension into the pre-chilled and labeled cryovial.

3.10 Place the cryovial in a CoolCell® Freezing Container or in a Styrofoam rack at - 80°C overnight, and transfer to liquid nitrogen the next day.