Applied StemCell, Inc.
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Datasheet

Genome Edited iPSCs
PARK2$^{+/-}$ Knockout

Product Information

Catalog Number	**ASE-9410**
Description	Applied StemCell's Genome Edited series of iPSC lines are ideal as *in vitro* models for neurodegenerative diseases such as Parkinson's Disease, Alzheimer's Disease, ALS, Autism and more. The ASE-9410 iPSC line is engineered with a mono-allelic (heterozygous) knockout for the PARK2 gene (PARK2$^{+/-}$) that has been implicated in Parkinson's disease. The parental iPSC is ASE-9109 which is an integration-free, normal karyotype iPSC derived from male, CD34+ cord blood cells. The PARK2 knockout line can be further differentiated into an isogenic panel of neurons and glia for disease modeling and drug/ toxicity screening applications.
Amount	≥1.0x10^6 viable cells/vial
Parental Cell Line	ASE-9109 (Male; control iPSC)
Gene Knockout	PARK2
Generated KO Line	PARK2 KO, clone G12F3
Type of KO	Mono-allelic (heterozygous)
Recovery	Plate entire contents of one vial onto one 60mm cell culture dish coated with Matrigel™
Culture Medium	mTeSR™1 Feeder-free Media
Shipping	Dry ice
Storage	Store in liquid nitrogen freezer immediately upon receipt.
Quality Control	Positive for pluripotency markers: OCT4, NANOG, TRA-1-81, TRA-1-60, and SOX2 Recovery of frozen cells: ≥70% viability
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. 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.

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Restricted Use This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Characterization of Genome Edited iPSC Lines

Expression of Pluripotency Markers

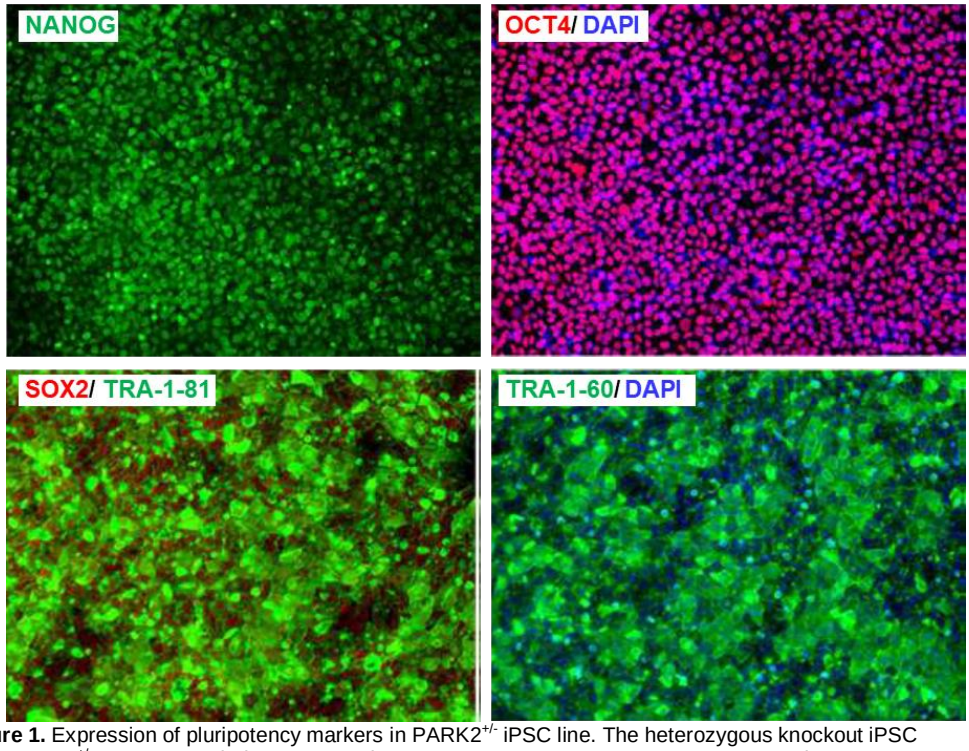

Figure 1. Expression of pluripotency markers in PARK2$^{+/-}$ iPSC line. The heterozygous knockout iPSC line, PARK2$^{+/-}$ expresses pluripotency markers NANOG, OCT4, SOX2, TRA-1-60, and TRA-1-81, indicating pluripotency of the iPSC line after genome editing. Nucleus stained with DAPI (blue).

Genotyping of PARK2$^{+/-}$ iPSC Line

PARK2 Heterozygous Knockout

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WT sequence:    GCCATGGTTTCccagtgGAGGTCGATTCT
Allele 1:       GCCATGGTTTCccagtgGAGGTCGATTCT
Allele 2:       GCCATGGTTT-----t-----TCGATTCT
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Allele 1: no mutation
Allele 2: deletion of 10 bp

Figure 2. Sequence alignment between wild type (WT) PARK2 sequence and PARK2$^{+/-}$ clone. The heterozygous knockout clone shows a 10 bp deletion in allele 2.

Additional Reagents Required but not Provided

- Rock Inhibitor, Reprocell, Cat# 04-0012
- mTeSR™1 Feeder Free Media, STEMCELL Technologies, Cat# 05850
- BD Matrigel™ hESC-qualified Matrix Features, BD Biosciences, Cat# 354277
- DMEM, Life Technologies, Cat# 11995081
- Accutase, Life Technologies, Cat# A1110501

- NutriFreez D10 Cryopreservation Medium, Reprocell, Cat# 01-0020-50

Equipment Required

Equipment	Specifications
Centrifuge	(e.g. Thermo Centra IEC CL2)
Conical Tubes	Polystyrene Conical Tube 15 mL, Corning Inc., Cat # 430790 Polystyrene Conical Tube 50 mL, Corning Inc., Cat# 176740
Cell Counter/Hemocytometer	
Cell Culture Dishes	60mm/15mm, Corning Inc., Cat# 430196

Protocol

All steps must be performed according to standards required for sterile cell culture work.

1. Handling Upon Receiving

Applied StemCell's Genome Edited iPSCs are shipped on dry ice at ambient temperature. Single or multiple vials with cryopreserved cells are packed in a transparent bag, which is buried in dry ice. Upon receiving the product, check the integrity of the packages and the presence of dry ice (contact Applied StemCell, if the integrity of a package has been compromised, e.g. no dry ice in the package).

Cells can be plated immediately after arrival or transferred into liquid nitrogen. Do not remove the vials from dry ice during transportation to storage units. Immediately transfer components (especially the cryopreserved iPSCs) to storage units, avoiding prolonged exposure to room temperature.

2. Coating Cell Culture Dishes or Flasks with BD Matrigel hESC-qualified Matrix Substrate

Please read producer's manual for handling BD Matrigel hESC-qualified Matrix.

Important producer's notes:

It is extremely important that BD Matrigel hESC- qualified Matrix and all culture ware or media coming in contact with Corning Matrigel hESC-qualified Matrix should be pre-chilled/ice-cold since BD Matrigel hESC-qualified Matrix will start to gel above 10°C.

The dilution is calculated for each lot based on the protein concentration. Prepare aliquots according to the dilution factor provided on the Certificate of Analysis (use 1.5-2.0 mL tubes). The volume of the aliquots is typically between 270-350 µL.

2.1 Pre-chill pipettes tips and dishes at 4°C.
2.2 Thaw an aliquot (typically between 270-350 µL) of BD Matrigel hESC-qualified Matrix at 4°C (approximately 45-60 minutes).
2.3 Transfer the aliquot on ice into biological safety cabinet.
2.4 Prepare a 25 mL aliquot of cold DMEM in 50 mL conical tube and keep on ice.
2.5 Using p1000 micropipette, transfer 1000 µL of the cold DMEM from the above tube into the tube with Matrigel and mix up and down several times. Transfer the Matrigel solution to the 50 mL conical tube containing cold DMEM and mix several times with a serological pipette (keep on ice).
2.6 Immediately, coat pre-chilled culture dishes with Matrigel/DMEM solution (volume 120-150 µL/cm^2).
2.7 Distribute coating matrix evenly and incubate at room temperature (15-25°C) for at least 1 hour before use.
2.8 Coated dishes can be used immediately or can be stored at 4°C for up to 7 days (aseptic conditions).

Table 1. Recommended volumes of coating reagents for various vessels.

Vessel	Approx. Surface Area	Matrigel
96 well plate	0.33 cm^2/well	50 µL/well
4 or 24 well plate	2 cm^2/well	250 µL/well
35 mm dish	10 cm^2	1.5 mL
60 mm dish	20 cm^2	2.5 mL

3. Recovering iPSC from Frozen Stock (Feeder free, Single Cell Suspension)

3.1 Pre-warm mTESR1 culture medium to 37°C and supplement the medium with 10 µm (final concentration of ROCK inhibitor).
3.2 Pre-warm Matrigel coated dishes.
3.3 Pipette out 5 mL of the above medium into a 15 mL conical tube.
3.4 Transfer a vial with frozen iPSC (on dry ice) to the operation side and thaw cells immediately in 37°C degree water bath until only a small piece of ice is still visible (approximately 1-1.5 minutes).
3.5 Transfer the vial to the biological safety cabinet and transfer thawed iPSC slowly and drop-wise into a 15 mL conical tube containing 5 mL of pre-warmed mTESR1 + ROCK medium under constant swirling.
3.6 Wash the cryo-vial with additional 1 mL of medium and transfer to the same 15 mL tube.
3.7 Centrifuge cells at 1000 rpm for 5 minutes.
3.8 Aspirate supernatant carefully without disturbing the pelleted cells and re-suspend the pellet carefully but thoroughly (5-6 up and down mixes) in 5 mL of fresh mTESR1 + ROCK medium using 5 mL serological or p1000 micro-pipette with a long tip.
3.9 Count the cells (optional).
3.10 Aspirate Matrigel solution from coated dishes and immediately seed iPSC at 8 x 10^4-15 x 10^4 cells/cm^2 (do not allow the matrix to dry out). We recommend seeding the entire vial onto one 60 mm dish.

Optional: *Aspirate Matrigel before the cells are thawed and add enough medium to cover the bottom of the culture dish.*

3.11 Distribute cells evenly by performing cross-like moves and place dish(es) in 37°C / 5°CO_2 cell culture incubator.
3.12 Change mTESR1 medium (without ROCK inhibitor) every day.

4. Enzymatic Passage of Feeder Free Cultured iPSC Using Accutase (Single Cell Suspension)

4.1 Pre-warm plates coated with Matrigel at 37°C.
4.2 Prepare new plate for transfer of clones: Remove the Matrigel solution from the plates and add appropriate volume of pre-warmed mTeSR1 supplemented with 10 µM ROCK inhibitor to each plate (5 mL for 60mm, 10 mL for 100mm plates, etc.).
4.3 Aspirate media from the plate to be passaged. Add pre-warmed Accutase to each plate (2.5 mL for 60mm, 5 mL for 100mm plates) and place the plate in a 37°C incubator. Observe intermittently to determine when cells begin to detach. Do not leave cells in Accutase for longer than 10 minutes.
4.4 When cells are detached, add an equal volume of pre-warmed DMEM to each plate to dilute the Accutase (2.5 mL for 60mm, 5 mL for 100mm plates).
4.5 Pipette the cell mixture in the plates up and down using a p1000 pipette. Wash the bottom of the plate well to ensure detachment of cells. Transfer the cell suspension to a conical tube.
4.6 Centrifuge tubes at 1000 rpm for 5 minutes.
4.7 Aspirate the supernatant and re-suspend cells thoroughly using p1000 pipette in mTESR1 (4 mL for pellet from 60mm dish, 9 mL for pellet from 100mm dish).
4.8 Centrifuge tube at 1000 rpm for 5 minutes.
4.9 Take 10 µL cell mixture, mix with 10 µL of Trypan blue and use for cell count.
4.10 Plate the cells on the previously prepared Matrigel coated plates with mTESR1 + ROCK medium at

densities ranging from 5 x 10^4 - 1 x 10^5 cells/cm^2.

4.11 Incubate at 37°C/ 5°CO_2 and observe attachment within 12 hours. Change media after 24 hours and daily thereafter.

5. Cryopreservation of iPSCs

5.1 Aspirate media from plates to be harvested. Add pre-warmed Accutase to each plate and place the plate in a 37°C incubator. Observe intermittently to determine when cells begin to detach (approximately 5 minutes). Do not leave cells in Accutase for longer than 10 minutes.

5.2 When cells are detached, add an equal volume of pre-warmed DMEM to each plate to dilute the Accutase, perform cell count and calculate how many vials need to be frozen down (e.g. 2.5 x 10^6 cells per vial).

5.3 Centrifuge the tube at 1000 rpm for 5 minutes.

5.4 Remove supernatant from tube and re-suspend cells in appropriate volume of cryopreservation media to achieve 2.5 x 10^6 cells per 0.5 mL.

5.5 Transfer 0.5 mL of the cell suspension in cryopreservation media to each cryo-vial and place the vials in a freezing container and immediately place the container at -80°C.

5.6 Transfer vials to LN2 within a week's time window.