Product Information and Testing for Depositor Material

Product Information

Product Name	LT2e-H9CAGGFP
WiCell Lot Number	DB0001
Depositor	Life Technologies
Banked by	Life Technologies
Thaw Recommendation	Thaw 1 vial into 2 wells of a 6 well plate.
Culture Platform	Feeder Dependent
	Medium: hES Medium
	Matrix: MEF
Protocol	Because this material was produced by the cell line depositor, WiCell recommends using the depositor protocol included in the CoA and testing results packet.
Passage Number	p68 These cells were cultured for 68 passages prior to freeze. The Jump-In R4 clone (C23) was derived from WA09 at p50 and the iCAGG clone was derived from C23 at p60. Add +1 to the passage number to best represent the overall passage number of the cells at thaw.
Date Vialed	06-April-2012
Vial Label	LT2e-H9CAGGFP P8 06-April-2012
Biosafety and Use Information	Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. WiCell is not responsible for damages or injuries that may result from the use of these cells. Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans.

Testing Performed by WiCell

Test Description	Test Provider	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	WiCell	SOP-CH-305	Recoverable attachment after passage	Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 16 HS System by Promega	Defines profile	Pass
Sterility	Biotest Laboratories	ST/07	Negative	Pass
Mycoplasma	WiCell	SOP-QU-004	Negative	Pass
Karyotype by G-banding	WiCell	SOP-CH-003	Report karyotype	Pass

Testing Performed by Depositor

No testing performed by Depositor.

Date of Lot Release	Quality Assurance Approval
04-April-2013	10/11/2016 X AMK AMK Quality Assurance Signed by: Klade, Anjelica

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Short Tandem Repeat Analysis*

Sample Report: 10702-STR Label on Tube: 10702-STR Sample Date: 02/08/13
Lab Received 02/08/13

Requestor: WiCell Research Institute

Test Date: 02/13/13 File Name: 130213 Report Date: 02/16/13

Sample Name: (label on tube) 10702-STR

Description: WI Cell Research Institute provided genomic DNA
239 ug/mL 260/280=2.01

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	12,13
D7S820	6-14	9,11
D13S317	7-15	9,9
D5S818	7-15	11,12
CSF1PO	6-15	11,11
TPOX	6-13	10,11
Amelogenin	NA	X,X
TH01	5-11	9.3,9.3
vWA	11, 13-21	17,17

Comments: Based on the 10702-STR DNA submitted by WI Cell dated and received on 02/08/13, this sample (Label on Tube: 10702-STR) defines the human stem cell line LT2e-H9CAGGFP comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human LT2e-H9CAGGFP stem cell line were detected and the concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. This result suggests that the 10702-STR DNA sample submitted corresponds to the LT2e-H9CAGGFP stem cell line and was not contaminated with any other human stem cells or a significant amount of mouse feeder layer cells. Sensitivity limits for detection of STR polymorphisms unique to either this or other human stem cell lines is ~5%.

[redacted]
Molecular Diagnostics Laboratory

[redacted]
Molecular Diagnostics Laboratory

* Testing to assess engraftment following bone marrow transplantation was accomplished by analysis of human genetic polymorphisms at STR loci. This methodology has not yet been approved by the FDA and is for investigational use only.

File: Final STR Report

Biotest Laboratories, Inc.

[redacted]
FDA Registered GMP | ISO 13485:2003 www.biotestlabs.com | ISO/IEC 17025:2005 EN/ISO 17665 | Phone: 763-315-1200 Fax: 763-315-1201

STERILITY REPORT

WiCell Research Institute, Inc.
WiCell Quality Assurance
[redacted]

BIOTEST SAMPLE # 13030760
VALIDATION # NG
TEST PURPOSE NG

PRODUCT NAME	Please see packing slip under product name.		
PRODUCT LOT	NA		
STERILE LOT	NA	BI LOT	NA
STERILIZATION LOT	NA	BI EXPIRATION DATE	NA
STERILIZATION DATE	NA	DATE RECEIVED	2013-03-15
STERILIZATION METHOD	NA	TEST INITIATED	2013-03-15
SAMPLING BLDG / ROOM	NA	TEST COMPLETED	2013-03-29

REFERENCE: Processed according to SOP LAB-003: Sterility Test Procedure.

11 products were divided between 40 mL TSB and 40 mL FTG. The samples were then cultured at 20-25 C and 30-35 C respectively and were monitored for a minimum of 14 days.

☒ USP
☐ BI Manufacturers Specifications
☐ Other

RESULTS	# POSITIVES	# TESTED	POSITIVE CONTROL	NEGATIVE CONTROL
☒ Sterile ☐ Non-Sterile ☐ NA	0	11	NA	2 Negatives

COMMENTS NA [redacted]

REVIEWED BY [redacted] DATE 29MAR13

WiCell Research Institute

Packing Slip

[redacted]

Sent to:
Sterility Testing Services
BiotestLabs, Sterility Testing Services
[redacted]

Date:
12Mar13

Product Name	Condition
[redacted] LT2e-H9CAGGFP #10731 [redacted]	-80

13030760 SLK
MAR 15 2013

Mycoplasma Report

Testing Performed by WiCell

RP/CDM-LRT #10702

FORM SOP-QU-004.01

Version B

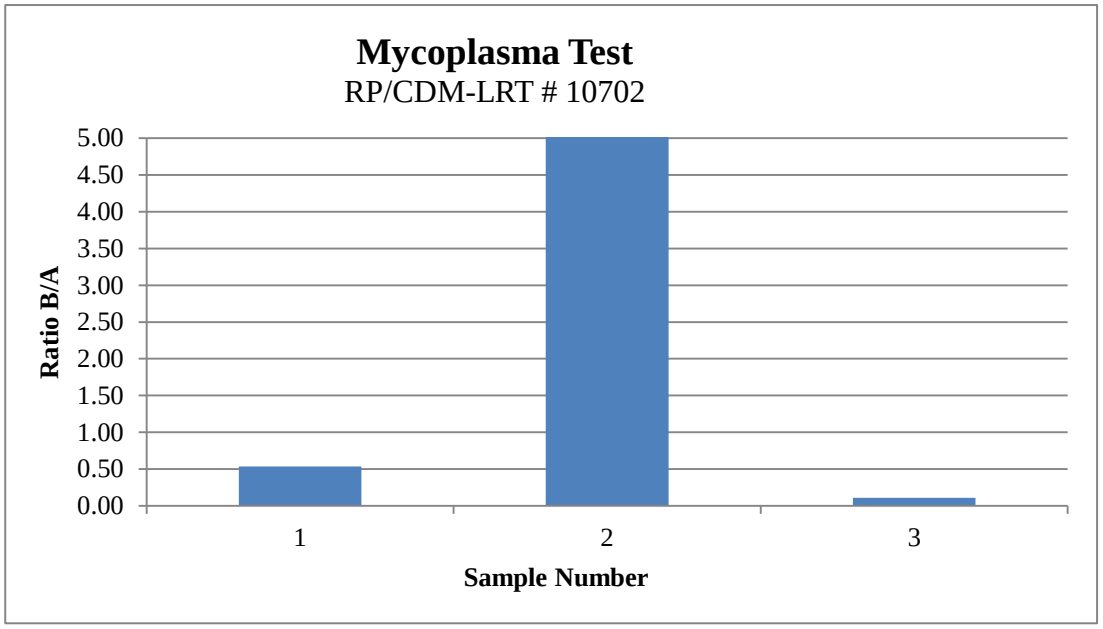
Edition 01

Assay performed and reported by: MW

Reviewed by: JB

Equipment ID: 539 Berthold

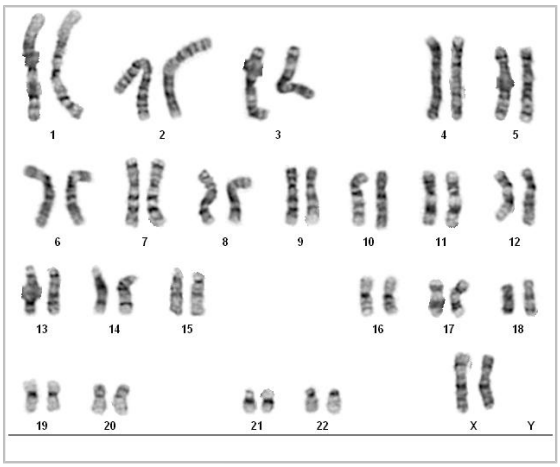
	Sample Number and ID	Reading A		A	Reading B		B	Ratio	Mycoplasma Results	Comments/Suggestions
		A1	A2	Average	B1	B2	Average	B/A		
1	LT2e-H9CAGGFP JB#10702	148	142	145	82	74	78	0.54	Negative	
2	Positive (+) Control	218	213	215.5	10470	10437	10453.5	48.51	Positive	
3	Negative (-) Control	347	351	349	39	38	38.5	0.11	Negative	



Chromosome Analysis Report: 009894

Date Reported: Wednesday, February 13, 2013
Cell Line: LT2e-H9CAGGFP 10702
Passage#: 12
Date of Sample: 2/6/2013
Specimen: hESC
Results: 46,XX

Cell Line Gender: Female
Reason for Testing: Lot release testing
Investigator: [redacted] CDM



Cell: 9
Slide: 2
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyotyped: 4
Band Resolution: 400 - 450

Interpretation:

This is a normal karyotype. No clonal abnormalities were detected at the stated band level of resolution.

Completed by: [redacted] CG(ASCP)
Reviewed and Interpreted by: [redacted], PhD, FACMG

A signed copy of this report is available upon request.

Date:_____ **Sent By:**____ **Sent To:**_____ **QC Review By:** ____

Limitations: This assay allows for microscopic visualization of numerical and structural chromosome abnormalities. The size of structural abnormality that can be detected is >3-10Mb, dependent upon the G-band resolution obtained from this specimen. For the purposes of this report, band level is defined as the number of G-bands per haploid genome. It is documented here as "band level", i.e., the range of bands determined from the four karyograms in this assay. Detection of heterogeneity of clonal cell populations in this specimen (i.e.,mosaicism) is limited by the number of metaphase cells examined, documented here as "# of cells counted".

Human PSC feeder-free culture protocol

Reagents and special tools:

1. StemPro® hESC SFM kit (Life Technologies#A1000701, which contains 500 ml DMEM/F12 with Glutamax, 10 ml STEMPRO® hESC Supplement, 50 ml 25% Bovine Serum Albumin)
2. Basic fibroblast growth factor (Life Technologies#PHG0026)
3. β-Mercaptoethanol (55 mM, Life Technologies#21985-023)
4. Geltrex™ (Life Technologies#12760-021)
5. Dispase (Life Technologies#17105-041)
6. DPBS without $CaCl_2$ and $MgCl_2$ (Life Technologies#14190-250)
7. Dimethyl sulfoxide (DMSO, Sigma-Aldrich#D2650)
8. StemPro EZPassge disposal stem cell passaging tool (Life Technologies#23181-010)
9. Cell scraper (BD Falcon#353085)
10. Knockout serum replacer (KSR, Life Technologies#10828-028)

Solution and medium preparation

Basic FGF Solution (10 µg/ml, For 1 ml)

Basic FGF	10 µg
PBS	996 µl
25% BSA	4 µl

Aliquot and store at -20°C for up to 6 months

Dispase Solution (1 mg/ml, For 50 ml)

Dispase	50 mg
DMEM/F12	50 ml

Sterilize through 0.22 µm filter and store at 4°C for up to 7 days.

StemPro hESC Medium (For 100ml)

DMEM-F12	90.8 ml
STEMPRO® hESC Supplement	2 ml
25% BSA	7.2 ml
β-Mercaptoethanol	182 µl
Basic FGF solution	80 µl

Add bFGF (final concentration 8 ng/ml) and β-Mercaptoethanol (final concentration 0.1 mM) at the time of medium change. Medium lasts for up to 7 days at 4°C.

Human PSC feeder-free culture protocol

StemPro cryo-preservation medium A

StemPro hESC Medium (50%) + KSR (50%)

StemPro cryo-preservation medium B

StemPro hESC Medium (80%) + DMSO (20%)

Make fresh cryo-preservation medium A and B.

Coating with Geltrex™

1. Thaw Geltrex™ at 2 to 8°C overnight.
2. Remove DMEM/F-12 from 2 to 8°C storage. Dilute Geltrex ™ with DMEM/F-12 (1:30) and mix gently.
3. Cover the whole surface of each culture plate and dishes with the Geltrex™ solution (1 ml for each well of 6-well plate, 1.5 ml for a 60-mm dish, and 3-4 ml for a 100 mm dish).
4. Seal each dish with parafilm to prevent from drying up, and incubate 1 hour at 37°C or overnight at 4°C.
5. If the coated plates or dishes are not used right away, store the coated plates or dishes at 2 to 8°C for up to 1 week. Transfer plates or dishes to a laminar flow hood and allow them to equilibrate to room temperate (about 1 hour) before use.

Thawing and plating human PSCs

1. Coat culture dishes with Geltrex at least 1 hour before thawing human PSCs. Wear eye protection as cryo-vials stored in the liquid phase of liquid nitrogen may accidentally explode when warmed.

   Note: One 60 mm coated dish for one vial of frozen human PSCs (frozen vial contains cells from ½ of 60mm dish).
2. Wear ultra low temperature cryo-gloves. Remove a cryo-vial of PSCs from the liquid nitrogen storage tank using metal forceps.
3. Roll the vial between your gloved hands until the outside is free of frost. This should take between 10-15 seconds.
4. Immerse the vial in a 37°C water bath without submerging the cap. Swirl the vial gently.
5. When only an ice crystal remains, remove the vial from the water bath.
6. Quickly remove the sticker or copy the information written on the vial in your notebook. The writing may come off the vial after spraying outside of vial with 70% ethanol.
7. Spray outside of the vial with 70% ethanol and place it in hood
8. Pipette cells gently into a sterile 15 ml conical tube using a 1ml pipette.
9. Add 1 ml pre-warmed StemPro hESC medium into the vial to collect resident cells.

11. Using a pipette to remove StemPro hESC medium from the vial and add it to the 15 ml conical tube drop-wise. While adding the medium, gently move the tube back and forth to mix the human PSCs. This reduces osmotic shock to the human PSCs.
12. Centrifuge PSCs at 200 x g for 5 minutes and aspirate the supernatant.
13. Re-suspend the cell pellet in 5 ml StemPro hESC medium.
14. Centrifuge PSCs at 200 x g for 5 minutes and aspirate the supernatant.
15. Re-suspend the cell pellet in 5 ml StemPro hESC medium.
16. Aspirate Geltrex solution and label the Geltrex coated 60 mm dish with the passage number from the vial, the date and your initials.
17. Slowly add the human PSC suspension into the dish.
18. Place the dish gently into the incubator and move the dish in several quick back-and-forth and side-to-side motions to disperse cells across the surface of the dish.
19. Replace spent medium daily. If feeding more than one dish, use a different pipette for each dish to reduce risk of contamination. Examine cells under a microscope and colonies may be very small in the first 2 days.
20. Observe PSCs every day and passage cells whenever the colonies are too big or crowded. The ratio of splitting depends on the total number of PSC colonies in culture plate or dish (Approximately 1:3 for human PSCs at the first time of recovery).

Passaging human PSCs

Note: This protocol is for the culture on culture dishes and is not for human PSCs cultured in flasks because it is difficult to remove differentiated colonies in a flask. Also, StemPro EZPassge disposal stem cell passaging tool and cell scraper cannot reach cells in flask.

1. Coat culture dishes with Geltrex at least 1 hour before passage. Warm appropriate amount of dispase solution and StemPro hESC medium to 37°C in a water bath.
2. Remove PSC plates and dishes from incubator. Label differentiated colonies with a microscope marker.
3. Aspirate the spent medium with a Pasteur pipette. Remove differentiated colonies with a Pasteur pipette by aspirating.
4. Gently add 1 ml pre-warmed dispase solution to each well of 6-well plate, 3 ml to each 60 mm dish or 4 ml to each 100 mm dish.
5. Incubate for 3-5 minutes at 37°C until the edges of cell colonies begin to curl off.
6. Aspirate dispase solution and rinse with DPBS two times gently. Add 1 ml pre-warmed StemPro hESC SFM to each well of 6-well plate, 2 ml to each 60 mm dish or 3 ml to each 100 mm dish.
7. Roll StemPro EZPassge disposal stem cell passaging tool across the entire dish or plate in one direction (left to right). **When rolling, overlap the next area with the area rolled previously.** Rotate the culture

dish or plate 90 degree, and roll StemPro EZPassge disposal stem cell passaging tool across the entire dish or plate. **When rolling, overlap the next area with the area rolled previously.** This procedure produces relatively uniform size of cell clumps.

8. Use a cell scraper to gently detach the cells off the surface of the plates and dishes.
9. Gently transfer cell clumps using a 5-mL pipette and place into a 15 or 50 ml conical tube.
   Note: Do not break cell clumps into small pieces.
10. Add 1 ml pre-warmed StemPro hESC SFM to each well of 6-well plate, 2 ml to each 60 mm dish or 3 ml to each 100 mm dish to collect residual cells and add cell suspension to the tube.
11. Aspirate Geltrex solution from Geltrex coated vessels. Add 2.5 ml pre-warmed StemPro hESC SFM into each well of 6-well plate, 5 ml into each 60 mm dish or 10 ml into each 100 mm dish.
12. **Gently shake the tube to distribute cell clumps evenly.** Add **appropriate amount** of PSC suspension into each well of culture plate or dish according to **split ratio**.
    Note: The split ratio is variable, though generally between 1:4 and 1:6. Occasionally cells will grow at a different rate and the split ratio will need to be adjusted. A general rule is to observe the last split ratio and adjust the ratio according to the appearance of the PSC colonies. If the cells look healthy and colonies have enough space, split using the same ratio, if they are overly dense and crowding, increase the ratio, and if the cells are sparse, decrease the ratio. Cells will need to be split every 4-6 days based upon appearance.
12. Place vessels gently in an incubator and move culture vessels in several quick back-and-forth and side-to-side motions to disperse cells across the surface of vessels.
13. Gently change media the next day to remove non-attached cells, and change StemPro hESC SFM every day thereafter.
14. Observe cells every day and passage cells whenever the colonies are too big or crowded (approximately every 3 to 4 days).

Cryo-preserving human PSCs cultured in StemPro hESC SFM

1. Warm appropriate amount of dispase and StemPro hESC medium to 37°C in a water bath.
2. Aspirate the medium and gently add 1 ml of dispase solution into each well of 6-well plate, 2 ml into a 60 mm dish and 4 ml into a 100 mm dish.
3. Incubate for 3-5 minutes at 37°C until the edges of cell colonies begin to curl off.
4. Aspirate dispase solution and rinse with DPBS two times gently, and then add 1 ml StemPro hESC medium into each well of 6-well plate, 2 ml into a 60 mm dish and 4 ml into a 100 mm dish.
5. Roll StemPro EZPassge disposal stem cell passaging tool across the entire dish or plate in one direction (left to right). **When rolling, overlap the next area with the area rolled previously.** Rotate the culture dish or plate 90 degree, and roll StemPro EZPassge disposal stem cell passaging tool across the entire dish

or plate. **When rolling, overlap the next area with the area rolled previously.** This procedure produces relatively uniform size of cell clumps.

6. Use a cell scraper to gently detach the cells off the surface of the plates and dishes.
7. Gently transfer cell clumps using a 5-mL pipette and place into a 15 or 50 ml conical tube.
8. Wash vessels with 3 ml of StemPro hESC medium to collect resident cells and add cell suspension to the tube.
9. Centrifuge at 200 x g for 5 minutes.
10. Gently aspirate supernatant and re-suspend the cells with 1 ml StemPro cryo-preservation medium A for all cells from one 60 mm dish.
11. Add same volume of StemPro cryo-preservation medium B drop-wise.
12. Allocate 1 ml cell suspension into each cryotube and freeze cells at -80 °C overnight in isopropanol chamber.
13. Transfer cells into liquid nitrogen tank next day for long term storage.

Change of PSCs cultured on mouse embryonic fibroblasts (MEFs) to StemPro hESC SFM

If human PSCs are maintained on MEFs, use the following protocol to switch PSCs to feeder-free condition.

1. Coat culture dishes with Geltrex at least 1 hour before experiment.
2. When PSCs cultured on MEFs in dish or plate reach 80-90% confluence, aspirate spent medium and rinse cells with PBS two times.
3. Add 1 ml StemPro hESC medium into each well of 6-well plate, 2 ml into a 60 mm dish and 4 ml into a 100 mm dish.
4. Roll StemPro EZPassge disposal stem cell passaging tool across the entire dish or plate in one direction (left to right). **When rolling, overlap the next area with the area rolled previously.** Rotate the culture dish or plate 90 degree, and roll StemPro EZPassge disposal stem cell passaging tool across the entire dish or plate. **When rolling, overlap the next area with the area rolled previously.** This procedure produces relatively uniform size of cell clumps.
5. Use a cell scraper to gently detach the cells off the surface of the plates and dishes.
6. Gently transfer cell clumps using a 5-mL pipette and place into a 15 or 50 ml conical tube.
7. Wash vessels with 3 ml of StemPro hESC medium to collect resident cells and add cell suspension to the tube.
8. Aspirate Geltrex solution from Geltrex coated vessels. Add 2.5 ml pre-warmed StemPro hESC SFM into each well of 6-well plate, 5 ml into each 60 mm dish or 10 ml into each 100 mm dish.

Human PSC feeder-free culture protocol

9. Gently shake the tube to distribute cell clumps evenly. Add appropriate amount of PSC suspension into each well of culture plate or dish according to split ratio **(**generally 1:3 to 1:4).
10. Place vessels gently in an incubator and move culture vessels in several quick back-and-forth and side-to-side motions to disperse cells across the surface of vessels.
11. Gently change media the next day to remove non-attached cells, and change StemPro hESC SFM every day thereafter.
12. Observe cells every day and passage cells whenever the colonies are too big or crowded (approximately every 3 to 4 days).

Note: The contaminated MEFs will die off after 2-3 passages in StemPro hESC SFM.