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H9 Cre-LoxP

This cell line utilizes a transgene integration strategy employing a Cre recombination mediated cassette exchange. It has a built-in double loxP recombination exchange cassette and was isolated by screening for transgene integration into a silencing-resistant site that can sustain GFP expression during differentiating into cells representing the three germ layers. With Cre-mediated recombination, the new transgene can replace the master vector at the same integration site, which guarantees the silence-resistant expression of the new transgene as that of GFP. The master hESC line constitutively expressing GFP can also be used to trace human cells in transplant experiments. The master vector was transfected into the hES cell line by electroporation and was constructed to drive GFP expression by CAG promoter and drives the hygromycin resistant gene by PGK promoter which are flanked by LoxP and lox2272 sites.

Alias	WA09
Cell Type	Modified Human ES
Culture Platform / Protocol	Feeder Independent - TeSR1 Medium
Disease Model	N/A
Genetic Modification Keyword	Other
NIH Registry Approved	Yes
Karyotype	46,XX
Blood Type	A+
Publication	Publication
Provider	University of Wisconsin (Zhang)

QTY	PRICE	Quantity	<input type="checkbox"/> Check if you received this cell line previously Why?
1 - 2	\$1,250.00/vial	1	ADD TO CART
3 - 4	\$1,125.00/vial		
5+	\$1,062.50/vial		

Related Products

[H9 inGFPPhES](#)
[WA09 \(mTeSR™ 1/Matrigel™ Platform\)](#)

Current Lot Information

Lot Number	Lot Description	Passage Number	Banked By	Product Information and Testing
MCB-01	Modified ES Cell; Cre-Lox site	22	WiCell	PDF

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Product Information

Product Name	H9 Cre-LoxP
Lot Number	WA09(LOXGFP)-MCB-01
Depositor	University of Wisconsin – Laboratory of Dr. Su-Chun Zhang
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 1 well of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: mTeSR1
	Matrix: Matrigel
Protocol	WiCell Feeder Independent Protocol
Passage Number	p22 These cells were cultured for 21 passages prior to freeze. WiCell adds +1 to the passage number at freeze so that the number on the vial best represents the overall passage number of the cells at thaw.
Date Viald	22-December-2008
Vial Label	WA09(LOXGFP)-MCB-1 p22 MW 27 DEC 08 SOPCC038A
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	≥ 15 Undifferentiated Colonies, ≤ 30% Differentiation	Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega	Consistent with known profile	Pass
Sterility - Direct transfer method	Apptec	30744	Negative	Pass
Mycoplasma	Bionique	M250	No contamination detected	Pass
Karyotype by G-banding	WiCell	SOP-CH-003	Normal karyotype	Pass

Amendment(s):

Reason for Amendment	Date
CoA updated to include copyright information.	See Signature
CoA updated for format changes, including adding fields of thaw recommendation, vial label, protocol, and banked by.	28-Jun-2013
CoA updated for correction of original date of lot release	20-Feb-2012
CoA updated for clarification of test specifications and product description, and removed text regarding technical services	05-Oct-2010
CoA updated for format changes, clarification of test specifications, test method, addition of test provider, culture platform, and electronic signature, and reference to WiCell instead of the NSCB	19-Aug-2010
Original CoA	02-Jun-2009

Date of Lot Release	Quality Assurance Approval
02-June-2009	12/30/2013 X AMC _____ AMC Quality Assurance Signed by [REDACTED]

Short Tandem Repeat Analysis*

Sample Report: 3811-STR

UW HLA#: 60323

Sample Date: 02/05/09

Received Date: 02/05/09

Requestor: WiCell Research Institute

Test Date: 02/09/09

File Name: 090210

Report Date: 02/16/09

Sample Name: (label on tube) 3811-STR

Description: DNA Extracted by WiCell
272.17 ug/mL; 260/280 = 1.92

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 DNA 3811-STR dated 02/05/09 and received on 02/05/09 from WI Cell, this sample (UW HLA# 60323) matches exactly the STR profile of the human stem cell line H9 comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H9 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. These results suggest that the 3811-STR DNA sample submitted corresponds to the H9 stem cell line and it 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%.

A

226-09

Manager Date

HLA/Molecular Diagnostics Laboratory

7/09

PhD, Director Date

HLA/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.



APPENDIX I

Document #: DCF3008A
Edition #: 06
Effective date: 9/17/2003
Title: DNA FLUOROCHROME ASSAY RESULTS

DNA-FLUROCHROME ASSAY RESULTS

Procedures 3008, 3009, 3011

Sample ID # 56366 M-250 Date Rec'd: 02/11/2009 P.O. #

Indicator Cells Inoculated: Date/Initials: 2/12/09 / JA

Fixation: Date/Initials: 2/16/09 / JA

Staining: Date/Initials: 2/16/09 / JA

TEST/CONTROL ARTICLE:

WA09(LOXGFP)-MCB-1-Bp27

LOT# NA

Wicell QA
WiCell Research Institute

505 S. Rosa Rd. Suite 120
Madison, WI 53719

Phone: 608-441-8019

Fax #: 608-441-8028

DNA FLUROCHROME ASSAY RESULTS:

X **NEGATIVE:** A reaction with staining limited to the nuclear region, which indicates no mycoplasmal contamination.

 POSITIVE: A significant amount of extranuclear staining which strongly suggests mycoplasmal contamination.

 INCONCLUSIVE:
 A significant amount of extranuclear staining consistent with low - level mycoplasmal contamination or nuclear degeneration.

 A significant amount of extranuclear staining consistent with bacterial, fungal or other microbial contaminant or viral CPE. Morphology not consistent for mycoplasmal contamination.

COMMENTS:

Date: 2/16/09 Results Read by: JA Date of Review: 2-17-09 Reviewed by: SCA



Document#: DCF3013D
 Edition#: 10
 Effective Date: 07/15/2003
 Title: M-250 FINAL REPORT SHEET

M-250 FINAL REPORT

Direct Specimen Culture
 Procedure 3008, 3011, 3013

TO: Wicell QA
 Wicell Research Institute

BTL SAMPLE ID#: 56366 P.O.#: DATE REC'D: 02/11/2009

TEST/CONTROL ARTICLE:

WA09 (LOXGFP) -MCB-1-Bp27

LOT#: NA

DIRECT CULTURE SET-UP (DAY 0)

DATE: 02/11/2009

INDICATOR CELL LINE (VERO)

SEE DNA FLUOROCHROME RECORD SHEET

				DATE
THIOGLYCOLLATE BROTH	DAY 7	+	⊖	<u>02/18/2009</u>
	DAY 28	+	⊖	<u>03/11/2009</u>
BROTH-FORTIFIED COMMERCIAL	DAY 7	+	⊖	<u>02/18/2009</u>
	DAY 28	+	⊖	<u>03/11/2009</u>
BROTH-MODIFIED HAYFLICK	DAY 7	+	⊖	<u>02/18/2009</u>
	DAY 28	+	⊖	<u>03/11/2009</u>
BROTH-HEART INFUSION	DAY 7	+	⊖	<u>02/18/2009</u>
	DAY 28	+	⊖	<u>03/11/2009</u>

(See Reverse)

Document#: DCF3013D
 Edition#: 10
 Effective Date: 07/15/2003
 Title: M-250 FINAL REPORT SHEET

SAMPLE ID#:	56366	AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>02/18/2009</u>
	DAY 14	+ ⊖	+ ⊖	<u>02/25/2009</u>
	DAY 21	+ ⊖	+ ⊖	<u>03/04/2009</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>02/18/2009</u>
	DAY 14	+ ⊖	+ ⊖	<u>02/25/2009</u>
	DAY 21	+ ⊖	+ ⊖	<u>03/04/2009</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>02/18/2009</u>
	DAY 14	+ ⊖	+ ⊖	<u>02/25/2009</u>
	DAY 21	+ ⊖	+ ⊖	<u>03/04/2009</u>
BROTH SUBCULTURES (DAY 7)		DATE: <u>02/18/2009</u>		
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>02/25/2009</u>
	DAY 14	+ ⊖	+ ⊖	<u>03/04/2009</u>
	DAY 21	+ ⊖	+ ⊖	<u>03/11/2009</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>02/25/2009</u>
	DAY 14	+ ⊖	+ ⊖	<u>03/04/2009</u>
	DAY 21	+ ⊖	+ ⊖	<u>03/11/2009</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>02/25/2009</u>
	DAY 14	+ ⊖	+ ⊖	<u>03/04/2009</u>
	DAY 21	+ ⊖	+ ⊖	<u>03/11/2009</u>

RESULTS: No detectable mycoplasmal contamination

3-11-09

Date

 Laboratory Director

M-250 Procedural Summary: The objective of this test is to ascertain whether or not detectable mycoplasmas are present in an *in vitro* cell culture sample, be it a primary culture, hybridoma, master seed stock or cell line. This procedure combines an indirect DNA staining approach to detect non-cultivable mycoplasmas with a direct culture methodology utilizing three different mycoplasma media formulations. The indirect approach involves the inoculation of the sample into a mycoplasma-free VERO (ATCC) indicator cell line and performing a DNA fluorochrome assay after 72-120 hours of incubation. The direct culture aspect of the test utilizes three different mycoplasma media including both broth and agar formulations. The sample is inoculated into each of the 3 broth formulations and also onto duplicate plates (0.1 mL/plate) for each of the 3 agar formulations. Subculture from broth to fresh agar plates is carried out after 7 days incubation. Agar plates are incubated aerobically and microaerophilically in order to detect any colony forming units morphologically indicative of mycoplasma contamination. Issuance of the final report with signature of the Scientific Director/Study Director signifies that the required controls were performed concurrently with the test sample(s) as detailed in the referenced SOPs and that all test conditions have been found to meet the required acceptance criteria for a valid test, including the appropriate results for the positive and negative controls.

Report Date: February 03, 2009

Case Details:

Cell Line: WA09(LOXGFP)MCB-1 (3811)

Passage #: 25

Date Completed: 2/3/2009

Cell Line Gender: Female

Investigator: National Stem Cell Bank

Specimen: hESC on MEF feeder

Date of Sample: 1/28/2009

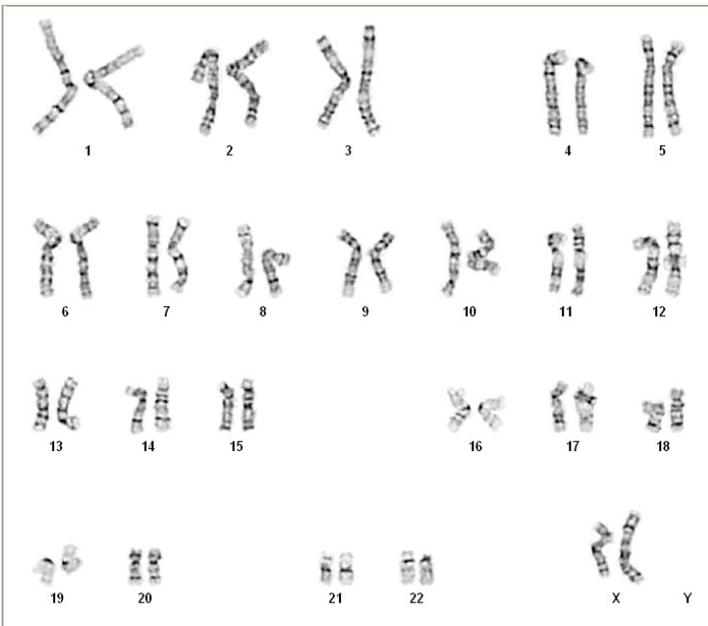
Tests, Reason for: FTDL

Results: 46,XX

Completed by _____, CLSp(CG), on 2/3/2009

Reviewed and interpreted by _____, PhD, FACMG, on 2/3/2009

Interpretation: No clonal abnormalities were detected at the stated band level of resolution.



Cell: S01-02

Slide: A

Slide Type: Karyotyping

Cell Results: Karyotype: 46,XX

of Cells Counted: 40

of Cells Karyotyped: 4

of Cells Analyzed: 7

Band Level: 450-500

Results Transmitted by Fax / Email / Post
Sent By: _____

Date: _____
Sent To: _____