



Product Information and Testing - Amended

Product Information

Product Name	WA13.C
Alias	H13
Lot Number	WB0054
Parent Material	WA13.C-DL-01
Depositor	University of Wisconsin – Laboratory of Dr. James Thomson
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 4 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: mTeSR1
	Matrix: Matrigel
Protocol	WiCell Feeder Independent Protocol
Passage Number	p10+21 These cells were cultured for 30 passages prior to freeze, 10 passages prior to subcloning, 20 after subcloning, and 4 of them in mTeSR1/Matrigel. 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	14-September-2010
Vial Label	WB0054 WA13.C p10+21 MW 14SEPT10
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, and removal of footnotes.	08-JUL-13
Original CoA	29-DEC-11

Date of Lot Release	Quality Assurance Approval
29-December-2010	<p style="text-align: right;">1/3/2014</p> <p style="text-align: center;">X AMC</p> <p>AMC Quality Assurance Signed by XXXXXXXXXX</p>



Short Tandem Repeat Analysis*

Sample Report: 4159-STR

UW HLA#: 63884

Sample Date: 10/01/10

Lab Received 10/01/10

Requestor: WiCell Research Institute

Test Date: 10/05/10

File Name: 101001SLE

Report Date: 10/06/10

Sample Name: (label on tube) 4159-STR

Description: DNA extracted by WiCell

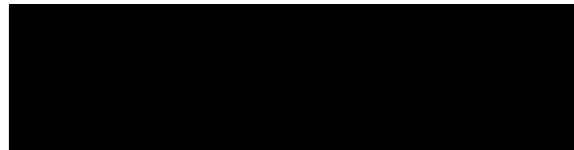
241.77ug/mL 260/280=1.93

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

Comments: Based on the 4159-STR DNA dated and received on 10/01/10 from WI Cell Research Institute, this sample (UW HLA# 63884) matches exactly the STR profile of the human stem cell line H13 comprising 14 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H13 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 4159-STR DNA sample submitted corresponds to the H13 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%.


Date

HLA/Molecular Diagnostics Laboratory


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.

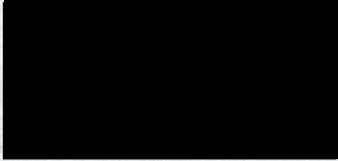
Test Facility:



This report is confidential. No part may be used for advertising or public announcement without written permission. Results apply only to the sample(s) tested.

Report Number
852056.A01
Page 1 of 1

WiCell Research Institute



December 1, 2010
P.O. #: [Redacted]
AMENDED REPORT
Original Issue Date:
11-27-10

❖ Amendment Summary

STERILITY TEST REPORT

Sample Information:

- hES Cells
- 1: WA15.07.07-WB0062 #1661
- 2: WA22-WB0046 #1491
- 3: WA13.C-WB0054 #7289
- 4: WA22-WB0053 #3855
- 5: iPS(IMR90)-3-WB0057 #3060
- 6: WA23-WB0067 #4696
- 7: WA15.07.03-WB0063 #8295

Date Received:

November 09, 2010

Date in Test:

November 11, 2010

Date Completed:

November 25, 2010

Test Information:

Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.201

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	0.5 mL	0.5 mL
Number Tested	14	14
Type of Media	SCD	FTM
Media Volume	400 mL	400 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	14 NEGATIVE	14 NEGATIVE

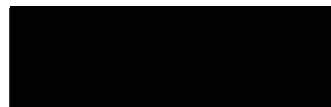
❖ A01 – Dated 12-01-10: Corrected sample information for sample # 1.



QA Reviewer

12-01-10

Date



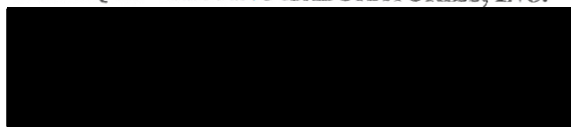
Technical Reviewer

12-01-10

Date

Testing conducted in accordance with current Good Manufacturing Practices.





APPENDIX

Document ID #: DCF9002E
Title: QUALITY ASSURANCE REPORT - GMP
Effective Date: 03/24/10
Edition #: 03

QUALITY ASSURANCE REPORT - GMP


<u>TEST PERFORMED</u>	<u>PROCEDURAL REFERENCE</u>	<u>TEST PERFORMED</u>	<u>PROCEDURAL REFERENCE</u>
<input checked="" type="checkbox"/> M-250	SOP's 3008, 3011, 3013	<input type="checkbox"/> M-700	SOP's 3008, 3009, 3010
<input type="checkbox"/> M-300	SOP's 3008, 3014	<input type="checkbox"/> M-800	SOP's 3008, 3011, 3016
<input type="checkbox"/> M-350	SOP's 3008, 3014, 3015		

Bionique Sample ID #(s) 102620

This testing procedure was performed in compliance with the FDA's Current Good Manufacturing Practice (cGMP) standards (to the extent that the regulations pertain to the procedures performed) as specified in the Code of Federal Regulations, Title 21 Parts 210 and 211 [21 CFR 210 & 211]. All related records derived from the test procedures have been reviewed by the Quality Assurance Department. The individual's signature below verifies that the methods and procedures referenced above have been followed and that the Final Report accurately reflects the raw data generated during the course of the procedures. All records, including raw data and final reports are archived on site for a minimum of seven years.

The specified test's procedures determine the intervals at which samples are inspected. The medium used for testing must pass quality control mycoplasmal growth promotion testing and sterility testing. Traceability of all of the components used is assured and supporting documentation can be supplied upon request.

Quality Assurance Review Date: 10/27/10

Reviewed By  QA Associate 

NOTE:

1. Prior to receipt at Bionique® Testing Laboratories, Inc., the stability of the test article is the responsibility of the company submitting the sample. Bionique Testing Laboratories Inc. will assume responsibility for sample stability following receipt and prior to being placed on test.
2. This test is for the detection of microbiological growth and does not require statistical validation.

Document ID #: DCF9002E
Title: QUALITY ASSURANCE REPORT - GMP
Effective Date: 03/24/10
Edition #: 03

REFERENCES

Regulatory:

1. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 210, Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General. FDA. Office of the Federal Register, National Archives and Records Department.
2. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 211, Current Good Manufacturing Practice for Finished Pharmaceuticals. FDA. Office of the Federal Register, National Archives and Records Department.
3. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, Director, Center for Biologics Evaluation and Research, FDA. May, 1993. Docket No. 84N-0154.
4. Department of Health and Human Services, Food and Drug Administration (USA) [FDA]. Code of Federal Regulations [CFR], Title 21 CFR Part 610.30, General Biological Products Standards; Subpart D, Test for Mycoplasma. FDA. Office of the Federal Register, National Archives and Records Department.

General:

1. Barile MF, Kern J. Isolation of Mycoplasma arginini from commercial bovine sera and its implication in contaminated cell cultures. Proceedings of the Society for Experimental Biology and Medicine, Volume 138, Number 2, November 1971.
2. Chen, T.R. In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Experimental Cell Research, 104: 255-262, 1977.
3. Carolyn K. Lincoln and Daniel J. Lundin. Mycoplasma Detection and Control. U. S. Fed. for Culture Collections Newsletter, Vol. 20, Number 4, 1990.
4. Fetal Bovine Serum; Proposed Guideline. National Committee For Clinical Laboratory Standards (NCCLS), Vol. 10, Number 6, 1990. (NCCLS publication M25-P).
5. McGarrity GJ, Sarama J, Vanaman V. Cell Culture Techniques. ASM News, Vol. 51, No. 4, 1985.
6. Tully JG, Razin S. Methods in Mycoplasma, Volumes I and II. Academic Press, N.Y., 1983.
7. Barile MF, Razin S, Tully JG, Whitcomb RF. The Mycoplasmas, Volumes 1-4. Academic Press, N.Y., 1979.
8. <http://www.bionique.com/> - Safe Cells Insights

MYCOPLASMA TESTING SERVICES

APPENDIX IV

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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#: 62620 P.O.#: DATE REC'D: 09/28/2010

TEST/CONTROL ARTICLE:

WA13.C-WB0054 #4159

LOT#: NA

DIRECT CULTURE SET-UP (DAY 0)

DATE: 09/29/2010

INDICATOR CELL LINE (VERO)

SEE DNA FLUOROCHROME RECORD SHEET

			DATE
THIOGLYCOLLATE BROTH	DAY 7	+ ⊖	<u>10/06/2010</u>
	DAY 28	+ ⊖	<u>10/27/2010</u>
BROTH-FORTIFIED COMMERCIAL			
<u>0.5</u> mL SAMPLE	DAY 7	+ ⊖	<u>10/06/2010</u>
<u>6.0</u> mL BROTH	DAY 28	+ ⊖	<u>10/27/2010</u>
BROTH-MODIFIED HAYFLICK			
<u>0.5</u> mL SAMPLE	DAY 7	+ ⊖	<u>10/06/2010</u>
<u>6.0</u> mL BROTH	DAY 28	+ ⊖	<u>10/27/2010</u>
BROTH-HEART INFUSION			
<u>0.5</u> mL SAMPLE	DAY 7	+ ⊖	<u>10/06/2010</u>
<u>6.0</u> mL BROTH	DAY 28	+ ⊖	<u>10/27/2010</u>

(See Reverse)

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

SAMPLE ID#: 62620		AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>10/06/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>10/13/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>10/20/2010</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>10/06/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>10/13/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>10/20/2010</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>10/06/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>10/13/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>10/20/2010</u>

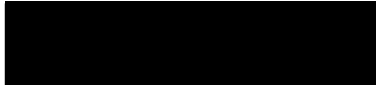
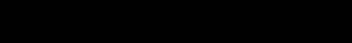
BROTH SUBCULTURES (DAY 7)

DATE: 10/06/2010

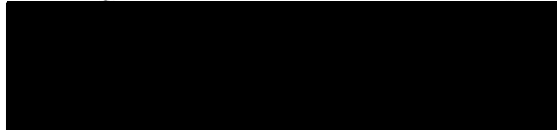
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>10/13/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>10/20/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>10/27/2010</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>10/13/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>10/20/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>10/27/2010</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>10/13/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>10/20/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>10/27/2010</u>

RESULTS: No detectable mycoplasmal contamination

10/27/10
 Date


 Laboratory Director
 Ph.D.

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 mycoplasmal 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 mycoplasmal 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 mycoplasmal contamination. Issuance of the final report with signature of the Laboratory 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.



Document ID #: DCF3008A
Title: DNA FLUOROCHROME ASSAY RESULTS
Effective Date: 3/24/10
Edition #: 07

DNA-FLUOROCHROME ASSAY RESULTS

Procedures 3008, 3009, 3011

Sample ID # 62620 M-250 Date Rec'd: 09/28/2010 P.O. #

Indicator Cells Inoculated: Date/Initials: 9/30/10 / HB

Fixation: Date/Initials: 10/4/10 / HB

Staining: Date/Initials: 10/4/10 / HB

TEST/CONTROL ARTICLE:

WA13.C-WB0054 #4159

LOT# NA

WiCell QA
WiCell Research Institute



DNA FLUOROCHROME ASSAY RESULTS:

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: 10/4/10 Results Read by: HB Date of Review: 10/4/10 Reviewed by: SM

Report Date: September 28, 2010

Case Details:

Cell Line: WA13.C-WB0054 (4159)

Passage #: 10+21

Date Completed: 9/28/2010

Cell Line Gender: Male

Investigator: Wisconsin International Stem Cell Bank

Specimen: hESC on Matrigel

Date of Sample: 9/20/2010

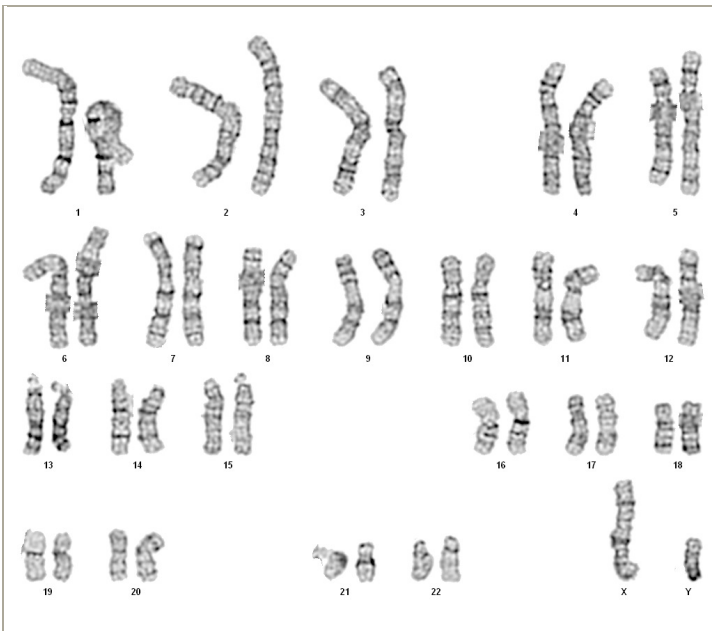
Tests, Reason for: lot release testing

Results: 46,XY

Completed by [REDACTED], CG(ASCP), on 9/23/2010

Reviewed and interpreted by [REDACTED] PhD, FACMG, on 9/28/2010

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



Cell: S01-05

Slide: 3-21

Slide Type: Karyotyping

of Cells Counted: 21

of Cells Karyotyped: 5

of Cells Analyzed: 9

Band Level: 450-475

Results Transmitted by Fax / Email / Post

Sent By: _____

QC Review By: _____

Date: _____

Sent To: _____

Results Recorded: _____