Product Information and Testing - Amended

Product Information

Product Name	MIRJT6i-mND1-4
Lot Number	WB0162
Depositor	University of Wisconsin – Laboratory of Dr. James Thomson
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 1 well of a 6 well plate. WiCell recommends thawing using ROCK Inhibitor for best results.
Culture Platform	Feeder Independent
	Medium: E8
	Matrix: Matrigel
Protocol	WiCell Feeder Independent E8 Medium Protocol
Passage Number	p30 These cells were cultured for 29 passages prior to freeze, at least 3 of them in E8/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 Vialed	21-May-2012
Vial Label	WB0162 MIRJT6i-mND1-4 p30 21MAY12 DF
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[1]
	[1]This test was the first STR performed for this cell line and therefore establishes the STR identity for this cell line.			
Sterility - Direct transfer method	Apptec	30744	Negative	Pass
Mycoplasma	Bionique	M250	No contamination detected	No contamination detected
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 incorporation of footnotes into the tables.	01-JUL-2013
Original CoA.	17-SEPT-2012

Date of Lot Release	Quality Assurance Approval
17-September-2012	12/31/2013 X AMC AMC Quality Assurance Signed by: [redacted]

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Short Tandem Repeat Analysis*

Sample Report: 10541-STR | Label on Tube: 10541-STR | Sample Date: 07/20/12
Lab Received 07/20/12

Requestor: WiCell Research Institute

Test Date: 07/25/12 | File Name: 120725 | Report Date: 07/27/12

Sample Name: (label on tube) 10541-STR

Description: WI Cell Research Institute provided genomic DNA
259 ug/mL 260/280=1.92

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	Identifying information has been redacted to protect donor confidentiality. If more information is required, please, contact WiCell's Technical Support.
D7S820	6-14	
D13S317	7-15	
D5S818	7-15	
CSF1PO	6-15	
TPOX	6-13	
Amelogenin	NA	
TH01	5-11	
vWA	11, 13-21	

Comments: Based on the 10541-STR DNA submitted by WI Cell dated and received on 07/20/12, this sample (Label on Tube: 10541-STR) defines the STR profile of the human stem cell line MIRJT6i-mND1-4 comprising 15 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human stem cell line MIRJT6i-mND1-4 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 suggest that the 10541-STR DNA sample submitted corresponds to the MIRJT6i-mND1-4 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

Test Facility:
[redacted]

WuXi AppTec

Report Number
907527
Page 1 of 1

WiCell Research Institute
[redacted]

August 30, 2012
P.O. #: [redacted]

STERILITY TEST REPORT

Sample Information:
1: DF19-9-7T-FTDL-01 10573
2: WA09-WB0156 10574
3: MIRJT6i-mND1-4-WB0163 10576
4: MIRJT6i-mND1-4-WB0162 10577
5: iPS(IMR90)-4-CB-01 10578
6: IISH6i-CML17-WB0170 10579
7: WA25-WB0169 10580

Date Received: August 09, 2012
Date in Test: August 15, 2012
Date Completed: August 29, 2012

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	12 NEGATIVE 2 POSITIVE	12 NEGATIVE 2 POSITIVE

Note: SCD and FTM Samples WA09-WB0156 10574 positive.

[redacted]
QA Reviewer **Date**

[redacted]
Technical Reviewer **Date**

Testing conducted in accordance with current Good Manufacturing Practices.

[redacted]

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APPENDIX

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

QUALITY ASSURANCE REPORT – GMP

TEST PERFORMED	PROCEDURAL REFERENCE	TEST PERFORMED	PROCEDURAL REFERENCE
☒ M-250	SOP's 3008, 3011, 3013	☐ M-700	SOP's 3008, 3009, 3010
☐ M-300	SOP's 3008, 3014	☐ M-800	SOP's 3008, 3011, 3016
☐ M-350	SOP's 3008, 3014, 3015		

Bionique Sample ID #(s) 70773

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: 8/15/12

Reviewed By [redacted] QA Associa[redacted]

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.

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 Mycoplasmology, 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

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APPENDIX IV

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
[redacted]

BTL SAMPLE ID#: **70773** P.O.#: DATE REC'D: **07/17/2012**

TEST/CONTROL ARTICLE:

MIRJT6i-MND1-4-WB0162-H #10541 p39

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0) DATE: **07/18/2012**

INDICATOR CELL LINE (VERO) SEE DNA FLUOROCHROME RECORD SHEET

			DATE
THIOGLYCOLLATE BROTH	DAY 7	+ ⊖	07/25/2012
	DAY 28	+ ⊖	08/15/2012
BROTH-FORTIFIED COMMERCIAL			
0.5 mL SAMPLE	DAY 7	+ ⊖	07/25/2012
6.0 mL BROTH	DAY 28	+ ⊖	08/15/2012
BROTH-MODIFIED HAYFLICK			
0.5 mL SAMPLE	DAY 7	+ ⊖	07/25/2012
6.0 mL BROTH	DAY 28	+ ⊖	08/15/2012
BROTH-HEART INFUSION			
0.5 mL SAMPLE	DAY 7	+ ⊖	07/25/2012
6.0 mL BROTH	DAY 28	+ ⊖	08/15/2012

(See Reverse)

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

SAMPLE ID#: **70773**		AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	07/25/2012
	DAY 14	+ ⊖	+ ⊖	08/01/2012
	DAY 21	+ ⊖	+ ⊖	08/08/2012
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	07/25/2012
	DAY 14	+ ⊖	+ ⊖	08/01/2012
	DAY 21	+ ⊖	+ ⊖	08/08/2012
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	07/25/2012
	DAY 14	+ ⊖	+ ⊖	08/01/2012
	DAY 21	+ ⊖	+ ⊖	08/08/2012
BROTH SUBCULTURES (DAY 7)		DATE: **07/25/2012**		
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	08/01/2012
	DAY 14	+ ⊖	+ ⊖	08/08/2012
	DAY 21	+ ⊖	+ ⊖	08/15/2012
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	08/01/2012
	DAY 14	+ ⊖	+ ⊖	08/08/2012
	DAY 21	+ ⊖	+ ⊖	08/15/2012
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	08/01/2012
	DAY 14	+ ⊖	+ ⊖	08/08/2012
	DAY 21	+ ⊖	+ ⊖	08/15/2012

RESULTS: No detectable mycoplasmal contamination

8/15/12
Date

[redacted]

ADDITIONAL COMMENTS:

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 microaerophillically 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.

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[redacted]

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 # **70773** **M-250** Date Rec'd: **07/17/2012** P.O. # NA 7/18/12 KG

Indicator Cells Inoculated: Date/Initials: 7/19/12 / KG

Fixation: Date/Initials: 7/23/12 / MK

Staining: Date/Initials: 7/23/12 / MK

TEST/CONTROL ARTICLE:

MIRJT6i-MND1-4-WB0162-H #10541 p39

LOT# **NA**

WiCell QA
WiCell Research Institute

[redacted]

[redacted]

DNA FLUOROCHROME 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: 7/23/12 Results Read by: MK Date of Review: 7/23/12 Reviewed by: LC

Chromosome Analysis Report: 008462

Report Date: June 27, 2012

Cell Line: MIRJT6i-mND1-4-WB0162 10541

Passage #: 34

Date of Sample: 6/18/2012

Date Completed: 6/27/2012

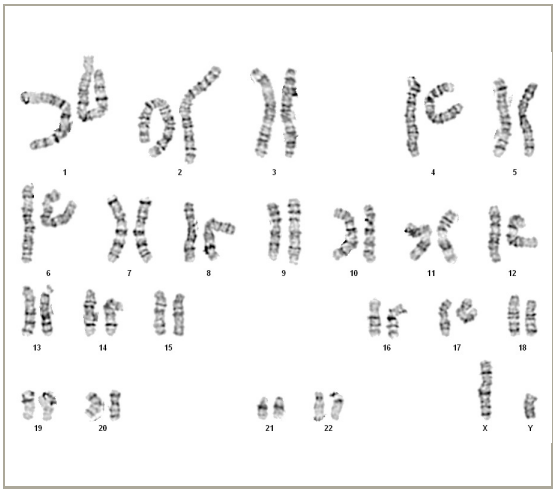
Results: 46,XY

Specimen: iPSC on Matrigel

Cell Line Gender: Male

Reason for Testing: Lot release testing

Investigator: [redacted]



Cell: S01-06

Slide: 1-R1 (4) karyotype

Slide Type: Karyotyping

# of Cells Counted: 20

# of Cells Karyotyped: 4

# of Cells Analyzed: 8

Band Level: 450-525

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

Completed by [redacted]**, CG(ASCP), on 6/27/2012**
Reviewed and interpreted by [redacted]**, PhD, FACMG, on 6/27/2012**

A signed copy of this report is available upon request.

Date:_____ **Sent To:**_____
Sent By:_____ **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".

This assay was conducted solely for listed investigator/institution. The results may not be relied upon by any other party without the prior written consent of the Director of the WiCell Cytogenetics Laboratory. The results of this assay are for research use only. If the results of this assay are to be used for any other purpose, contact the Director of the WiCell Cytogenetics Laboratory.