



**Product Information**

Product Name	WA17
Lot Number	WA17-pMCB-04
Parent Material	This material descended from derivation
Depositor	WiCell
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 3 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: mTeSR1
	Matrix: Matrigel
Protocol	WiCell Feeder Independent Protocol
Passage Number	p9  These cells were cultured for 8 passages prior to freeze. Cells were derived in Conditioned Medium on Matrigel. They were transitioned to mTeSR1 at passage 4 and cultured 4 additional 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-March-2010
Vial Label	WA17-pMCB-04 p09 DF 22MARCH2010 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.

**Lot Specific Testing Performed by WiCell**

The following tests were performed on this specific lot.

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



## Product Information and Testing - Amended

### General Cell Line Testing Performed by WiCell

The following tests were performed on the cell line. The tests do not apply to any particular lot.

Test Description	Test Provider	Test Method
Differentiation Potential by Teratoma	WiCell	SOP-CH-213 SOP-CH-214
HLA	UW Molecular Diagnostics Laboratory	PowerPlex 1.2 System by Promega
ABO	American Red Cross	For ABO: Olsson ML, Chester MA. A rapid and simple ABO genotype screening method using a novel B/O2 versus A/O2 discriminating nucleotide substitution at the ABO locus. Vox Sang 1995; 69(3):242-7. For RHD: Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, Narter-Olaga EG, Hawthorne LM, Daniels G. The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood 2000; 95(1): 12-8.
Growth Curve (Doubling Time)	WiCell	Varies by culture platform
Flow Cytometry for ESC Marker Expression	UW Flow Cytometry Laboratory	SOP-CH-101 SOP-CH-102 SOP-CH-103 SOP-CH-105
Array Comparative Genomic Hybridization (aCGH)	WiCell	SOP-CH-308 SOP-CH-309 SOP-CH-310
Comprehensive Human Virus Panel	Charles River	ID 91/0

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. General Cell Line Testing CoA added to lot CoA.	24-JUN-2013
Original CoA	17-MAR-2011

Date of Lot Release	Quality Assurance Approval
17-March-2011	<div style="text-align: right;">1/3/2014</div> <p><b>X</b> AMC _____            AMC            Quality Assurance            Signed by: _____</p>

## Short Tandem Repeat Analysis\*

Sample Report: 2148-STR

UW HLA#: 63255

Sample Date: 06/04/10

Received Date: 06/04/10

Requestor: WiCell Research Institute

Test Date: 06/08/10

File Name: 100609

Report Date: 06/13/10

Sample Name: (label on tube) 2148-STR

Description: DNA Extracted by WiCell  
257.89 ng/ $\mu$ L; 260/280 = 1.90

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	11,13
D7S820	6-14	8,12
D13S317	7-15	8,8
D5S818	7-15	12,12
CSF1PO	6-15	12,13
TPOX	6-13	11,11
Amelogenin	NA	X,Y
TH01	5-11	6,7
vWA	11, 13-21	17,19

**Comments:** Based on the 2148-STR DNA dated and received on 06/04/10 from the WiCell Research Institute, this sample (UW HLA# 63255) exactly matches the STR profile of the human embryonic stem cell line WA17 comprising 13 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA17 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 2148-STR DNA sample submitted 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%.

6-14-10

Date

HLA/Molecular Diagnostics Laboratory

6/14/10

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.

WiCell Research Institute

Report Number  
833787  
Page 1 of 1

April 26, 2010  
P.O. #: [REDACTED]

## STERILITY TEST REPORT

**Sample Information:**

- hES Cells  
 1: WA17-pMCB-03 # 9794  
 2: WA17-pMCB-04 # 2169  
 3: TE04-MCB-02 # 9051  
 4: ES01-DL-02 # 0431  
 5: ES06-DL-06 # 0142  
 6: WA01-DL-09 # 2852  
 7: WA01-DL-10 # 4205  
 8: WA01-DL-11 # 7858  
 9: WA01-DL-12 # 6048

**Date Received:**

April 02, 2010

**Date in Test:**

April 07, 2010

**Date Completed:**

April 21, 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	18	18
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	18 NEGATIVE	18 NEGATIVE

QA Reviewer

Date

Technical Reviewer

Date

Testing conducted in accordance with current Good Manufacturing Practices.





MYCOPLASMA TESTING SERVICES

APPENDIX

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Document ID #: DCF9002F  
Title: **QUALITY ASSURANCE REPORT - GMP**  
Effective Date: 03/12/10  
Edition #: 01

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**QUALITY ASSURANCE REPORT - G M P**

<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) 61143

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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: 6/9/10

Reviewed By \_\_\_\_\_, QA Assistant: \_\_\_\_\_

**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.

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Document ID #: DCF9002F  
Title: QUALITY ASSURANCE REPORT - GMP  
Effective Date: 03/12/10  
Edition #: 01

---

## 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

Page 1 of 2

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#: **61143** P.C

DATE REC'D: **05/11/2010**

TEST/CONTROL ARTICLE:

**WA17pMCB4 #2148**

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0)

DATE: **05/12/2010**

INDICATOR CELL LINE (VERO)

SEE DNA FLUOROCHROME RECORD SHEET

				DATE
THIOGLYCOLLATE BROTH	DAY 7	+	⊖	<b><u>05/19/2010</u></b>
	DAY 28	+	⊖	<b><u>06/09/2010</u></b>
BROTH-FORTIFIED COMMERCIAL				
<b><u>0.5</u></b> mL SAMPLE	DAY 7	+	⊖	<b><u>05/19/2010</u></b>
<b><u>6.0</u></b> mL BROTH	DAY 28	+	⊖	<b><u>06/09/2010</u></b>
BROTH-MODIFIED HAYFLICK				
<b><u>0.5</u></b> mL SAMPLE	DAY 7	+	⊖	<b><u>05/19/2010</u></b>
<b><u>6.0</u></b> mL BROTH	DAY 28	+	⊖	<b><u>06/09/2010</u></b>
BROTH-HEART INFUSION				
<b><u>0.5</u></b> mL SAMPLE	DAY 7	+	⊖	<b><u>05/19/2010</u></b>
<b><u>6.0</u></b> mL BROTH	DAY 28	+	⊖	<b><u>06/09/2010</u></b>

(See Reverse)

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

SAMPLE ID#:	61143	AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>05/19/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>05/26/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>06/02/2010</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>05/19/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>05/26/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>06/02/2010</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>05/19/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>05/26/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>06/02/2010</u>

**BROTH SUBCULTURES (DAY 7)**DATE: 05/19/2010

AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>05/26/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>06/02/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>06/09/2010</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>05/26/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>06/02/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>06/09/2010</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>05/26/2010</u>
	DAY 14	+ ⊖	+ ⊖	<u>06/02/2010</u>
	DAY 21	+ ⊖	+ ⊖	<u>06/09/2010</u>

RESULTS: No detectable mycoplasmal contamination

6/9/10  
 Date

**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 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.

MYCOPLASMA TESTING SERVICES

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 # 61143 M-250 Date Rec'd: 05/11/2010 P.O. #

Indicator Cells Inoculated: Date/Initials: 5/13/10 / KG

Fixation: Date/Initials: 5/17/10 / HS

Staining: Date/Initials: 5/17/10 / HS

TEST/CONTROL ARTICLE:

WA17pMCB4 #2148

LOT# NA

5/28/10 @  
w. cell out  
~~Distribution Lab~~  
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: 5/17/10 Results Read by: HS Date of Review: 5/17/10 Reviewed by: SAH



**Report Date:** June 03, 2010

**Case Details:**

**Cell Line:** WA17-pMCB-04 (7437)

**Passage #:** 12

**Date Completed:** 6/3/2010

**Cell Line Gender:** Male

**Investigator:**

**Specimen:** hESC on Matrigel

**Date of Sample:** 5/24/2010

**Tests, Reason for:** WB testing \*?Y chromosome

**Results:** 46,XY

**Completed by** MS, CG(ASCP), on 6/1/2010

**Reviewed and interpreted by** PhD, FACMG, on 6/3/2010

**Interpretation:** No clonal abnormalities were detected at the stated band level of resolution. There is no evidence for abnormality of the Y chromosome.



**Cell:** S01-01

**Slide:** A-8

**Slide Type:** Karyotyping

**# of Cells Counted:**

**# of Cells Karyotyped:**

**# of Cells Analyzed:**

**Band Level:**

**Results Transmitted by Fax / Email / Post**

**Sent By:** \_\_\_\_\_

**QC Review By:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Sent To:** \_\_\_\_\_

**Results Recorded:** \_\_\_\_\_

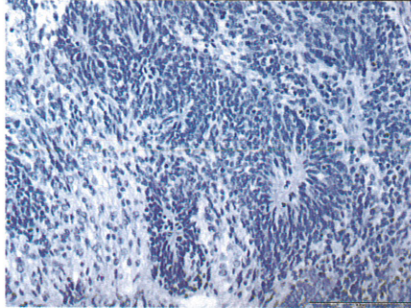
Cell Line: WA17-A

Cell Lot Number: pMCB-04

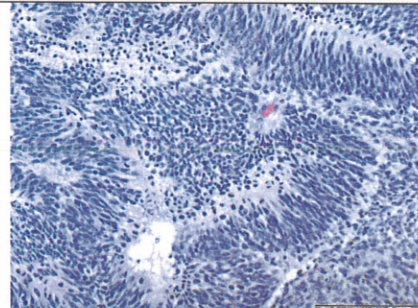
Sample Number: 3127A

**ECTODERM**

Structure Name: Neuroectoderm Magnification: 100x Slide ID: A

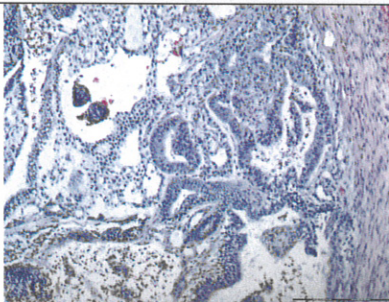


Structure Name: Neuroectoderm Magnification: 200X Slide ID: A

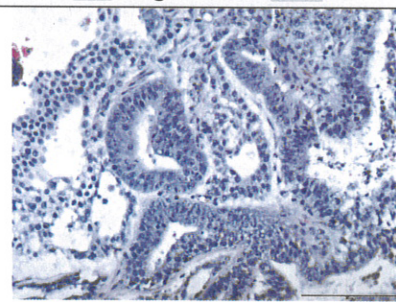


**ENDODERM**

Structure Name: Gut Magnification: 100X Slide ID: A

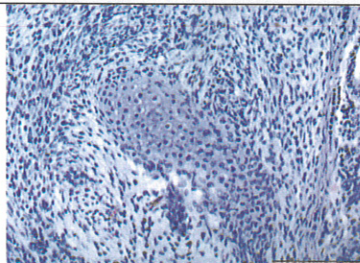


Structure Name: Gut Magnification: 200X Slide ID: A

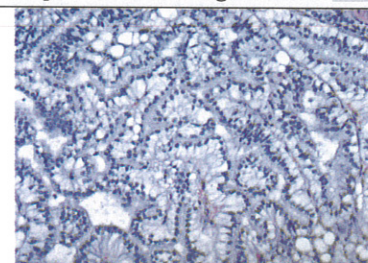


**MESODERM**

Structure Name: Cartilage Magnification: 200X Slide ID: A



Structure Name: Nephroid tissue Magnification: 200X Slide ID: A



Comments: Structures found correspond to Ectoderm (1), Mesoderm (3) and Endoderm (1)

Sample(s) were assessed for the presence of differentiation into cell types characteristic of the three embryonic germ layers, which, if present in the sample(s) examined, are represented in the photographs above. The individual's signature below verifies that this report accurately reflects the pathology observed.

Pathologist (By/Date):

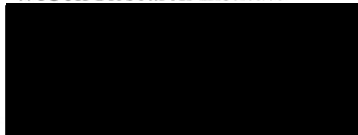
QA Review (By/Date):





Date: 03/17/2010 09:55:55

To: WiCell Research Institute



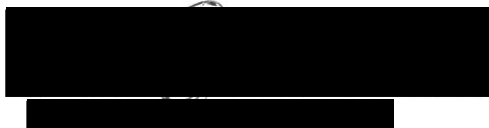
Re: High-resolution HLA results

**Patient**

Name HLA / MR# received	Dates		HLA DNA-based typing*							
			Method: PCR-SSP			Direct Sequencing				PCR-SSP
			A*	B*	C*	DRB1*	DRB3*	DRB4*	DRB5*	DQB1*
WICELL, 5391-HLA	DQB SSP		1101	1501/38	0303/20N	1104				
62664 /	A,B,C SSP	03/09/2010	2402	1801/11/1 7N	1203	1201/6/10 /17				
03/09/2010	DRB Seq	03/09/2010								



HLA/Molecular Diagnostics Laboratory

3/17/10 [signature]  
Date

HLA/Molecular Diagnostics Laboratory

03/18/10  
Date





**American  
Red Cross**

National Molecular Blood Group  
and Platelet Testing Laboratory



03/23/10

**SAMPLE:** 5391-ABO (ML10-0393)

Date received: 03/12/10

Sample date: 03/01/10

**INSTITUTION:** WiCell Research Institute/National Stem Cell Bank (WICELL)

**HISTORY:** DNA sample from cell line.

**TESTING REQUESTED:** Genotype for *ABO* and *RH*

**DNA TESTING PERFORMED:** ABO: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) testing for nucleotide positions 261 ( $O^1$ ), 467 ( $A^2$ ), 703 (B), and 1096 (B and  $O^2$ ). RH: PCR-multiplex analysis for *RHD* exons 4, 7, the inactivating *RHD* pseudogene and C/c genotyping. RHCE: PCR-RFLP for e/E in exon 5 (676G>C).

**DNA MOLECULAR RESULTS:**

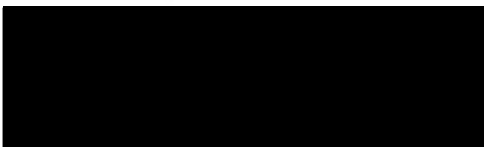
Genotype

Predicted Phenotype

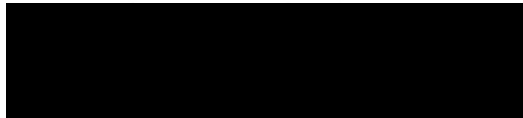
**5391-ABO:** *ABO\*O<sup>1</sup>O<sup>1</sup>; RHD, RHC, RHc, RHe*

**Group O; RhD+, C+, c+, E-, e+**

**RH COMMENTS:** The sample was negative for the *RHD*-inactivating pseudogene.



Scientific Director

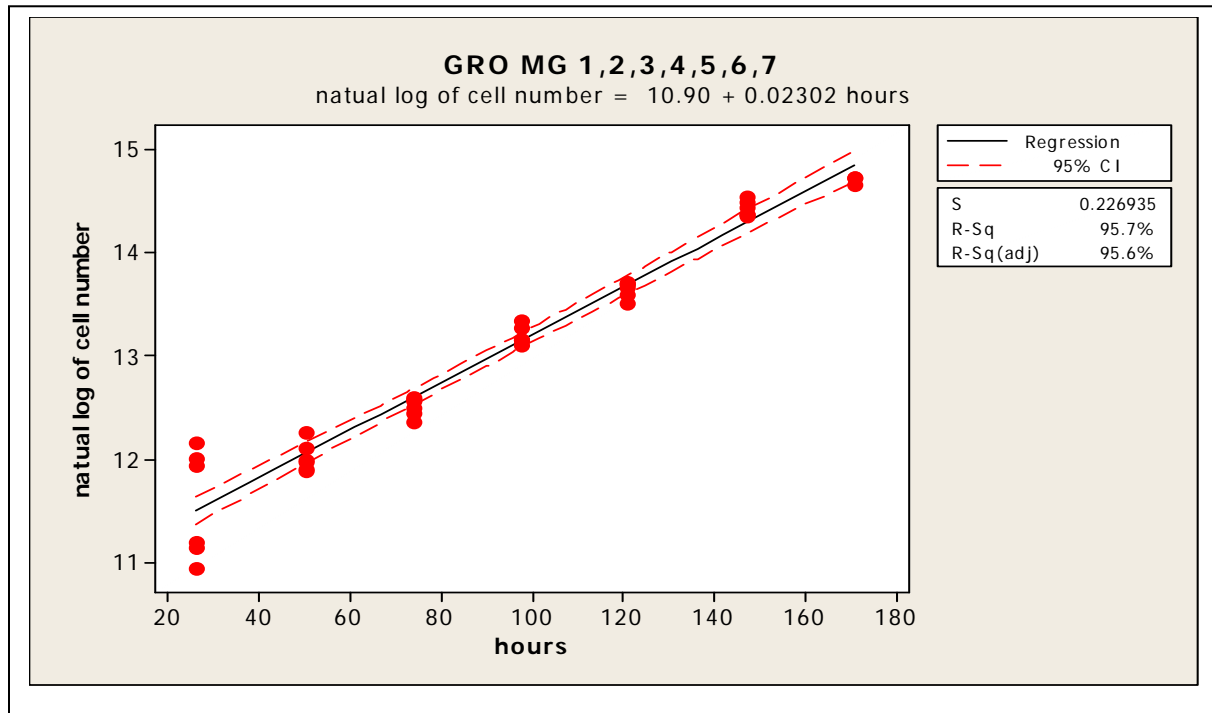


Supervisor

THE MOLECULAR TEST METHODS WERE DEVELOPED, AND THEIR PERFORMANCE CHARACTERISTICS DETERMINED BY THE MOLECULAR RED CELL AND PLATELET TESTING LABORATORY AT THE AMERICAN RED CROSS PENN-JERSEY REGION. THE FDA HAS NOT REVIEWED OR APPROVED THE REAGENTS USED. THESE RESULTS ARE NOT INTENDED AS THE SOLE MEANS FOR CLINICAL DIAGNOSIS OR PATIENT MANAGEMENT DECISIONS. **LIMITATIONS:** The genotype may not always reflect the red cell phenotype. New mutations that inactivate gene expression or rare new variant alleles may not be identified in these assays.

**Please Give Blood.**

Cell Line Information		QA Use
Sample ID: MG-TeSR - GRO 1,2,3,4,5,6,7	Cell lot #: WA17 in mTeSR1	Report reviewed by: JKT
Cell Line: WA17	Report prepared by: JB/MW	Report reviewed on: 10May10
Passage: p9	Date cells received: 3-23-2010	



<p><b>Regression Analysis: natural log of cell number versus hours</b></p> <p>The regression equation is natural log of cell number = 10.9 + 0.0230 hours</p> <table border="1"> <thead> <tr> <th>Predictor</th> <th>Coef</th> <th>SE Coef</th> <th>T</th> <th>P</th> </tr> </thead> <tbody> <tr> <td>Constant</td> <td>10.9036</td> <td>0.0823</td> <td>132.49</td> <td>0.000</td> </tr> <tr> <td>hours</td> <td>0.0230186</td> <td>0.0007986</td> <td>28.82</td> <td>0.000</td> </tr> </tbody> </table> <p>S = 0.226935 R-Sq = 95.7% R-Sq(adj) = 95.6%</p> <p>Analysis of Variance</p> <table border="1"> <thead> <tr> <th>Source</th> <th>DF</th> <th>SS</th> <th>MS</th> <th>F</th> <th>P</th> </tr> </thead> <tbody> <tr> <td>Regression</td> <td>1</td> <td>42.789</td> <td>42.789</td> <td>830.87</td> <td>0.000</td> </tr> <tr> <td>Residual Error</td> <td>37</td> <td>1.905</td> <td>0.051</td> <td></td> <td></td> </tr> <tr> <td>Total</td> <td>38</td> <td>44.695</td> <td></td> <td></td> <td></td> </tr> </tbody> </table>		Predictor	Coef	SE Coef	T	P	Constant	10.9036	0.0823	132.49	0.000	hours	0.0230186	0.0007986	28.82	0.000	Source	DF	SS	MS	F	P	Regression	1	42.789	42.789	830.87	0.000	Residual Error	37	1.905	0.051			Total	38	44.695				<p><b>Slope ± 95% C.I. 0.025 ± 0.0016</b></p> <p><b>Doubling Time ± 95% C.I.</b></p> <p><b>32.1 hours ± 2.3 hours</b></p> <p><b>29.9 hours - 32.3 hours</b></p>
Predictor	Coef	SE Coef	T	P																																					
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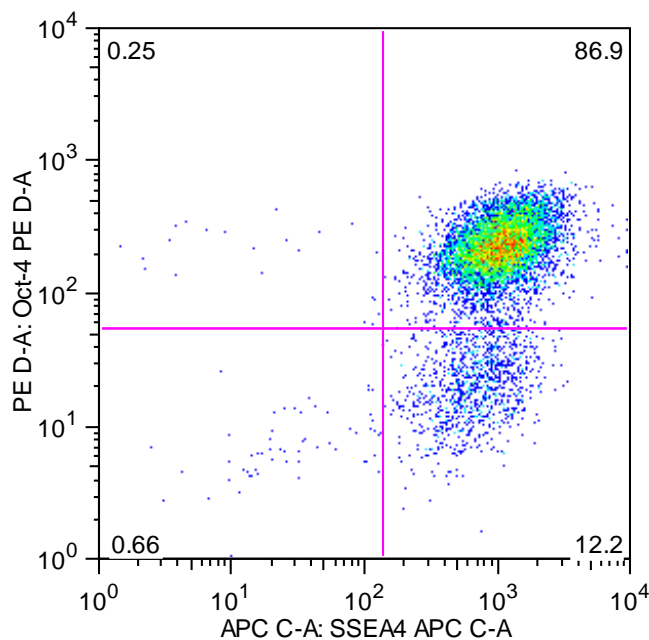
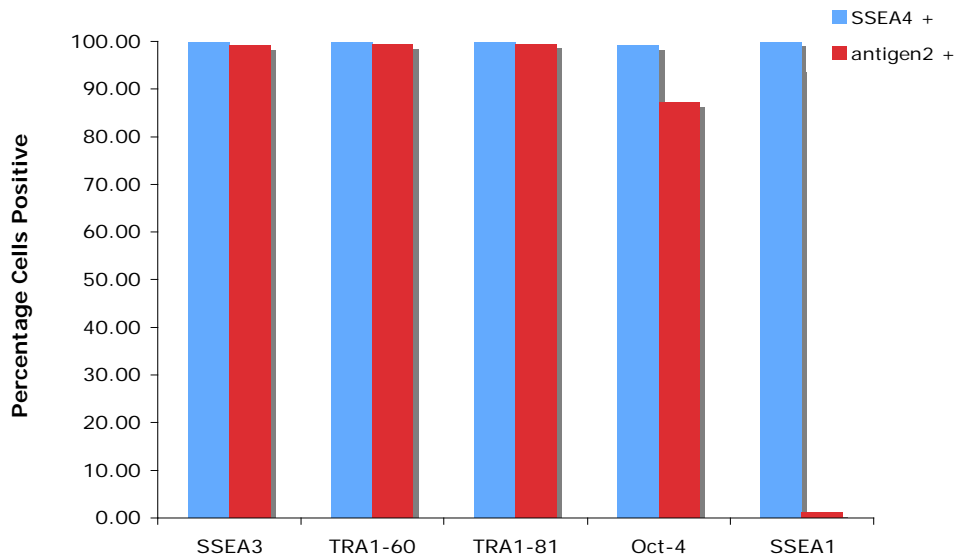




Cell Line Information		QA Use
Sample ID: MG-TeSR - GRO 1,2,3,4,5,6,7	Cell lot #: WA17 in mTeSR1	Report reviewed by: JKT
Cell Line: WA17	Report prepared by: JB/MW	Report reviewed on: 10May10
Passage: p9	Date cells received: 3-23-2010	

Photo Day 0- Colonies before splitting Pictures are not a part of the growth curve protocol for new derivations per MW. JKT 10May10	Photo Day 1
Photo Day 2	Photo Day 3
Photo Day 4	Photo Day 5

	SSEA4 -	SSEA4 +	SSEA4 +	SSEA4 -	ALL	ALL
<u>antigen2:</u>	<u>antigen2 +</u>	<u>antigen2 +</u>	<u>antigen2 -</u>	<u>antigen2 -</u>	<u>SSEA4 +</u>	<u>antigen2 +</u>
SSEA3	0.05	99.10	0.74	0.13	99.84	99.15
TRA1-60	0.13	99.10	0.67	0.07	99.77	99.23
TRA1-81	0.05	99.40	0.50	0.02	99.90	99.45
Oct-4	0.25	86.90	12.20	0.66	99.10	87.15
SSEA1	0.00	1.04	98.80	0.12	99.84	1.04



CD29-  
 1017\_test.fcs  
 Event Count: 8656

**Report Date:** 06/02/2011  
**Date of Sample:** 5/12/2010  
**Investigator:** ██████████  
**Reason for Testing:** New Derivation  
**Specimen:** hESC on Matrigel, TeSR  
**Karyotype Results:** 46,XY

**Test:** WA17-pMCB-03-A (Male)  
**Reference:** WA09-MCB-01-E.3-p19(2) (Female)  
**Project:** ██████████  
**Funding:** ██████████  
**CGH Accession #:** 000380  
**GEO Accession #:**

**Microarray Results:**

- arr(1-22,X)x2 – Female**
 **arr(1-22)x2,(XY)x1 – Male**
 **Consistent with a Balanced Karyotype (Karyotype Unavailable)**
- Consistent with the Karyotype Results**
 **Inconsistent with the Karyotype Results**
 **Additional Findings**

**Interpretation:**

**CNV gains/losses**

- There were **46** copy number gains and losses identified, including **2** pseudoautosomal regions and **13** copy number changes due to the reference DNA
- Select CNVs are detailed in the table below

Chr	Band (Genomic Position)	Width	Aberration Type	Classification	Genes
3*	arr 3p14.1(65,097,160-65,209,465)x3	112,304	Gain	Uncertain Significance – Likely Benign	
7	arr 7q22.1(101,904,922-102,064,178)x3	159,255	Gain	Uncertain Significance – Likely Benign	LRWD1, MGC119295, POLR2J, POLR2J2, POLR2J3, RASA4
7	arr 7q35(143,528,320-143,705,123)x1	176,803	Loss	Uncertain Significance – Likely Benign	<u>ARHGEF5</u> , FLJ43692, OR2A1, OR2A42, OR2A7
10	arr 10q11.21(43,028,823-43,249,649)x1	220,825	Loss	Uncertain Significance – No Sub-Classification	FXVD4, HNRPF, RASGEF1A
11	arr 11p11.12(49,065,902-49,931,312)x3	865,409	Gain	Uncertain Significance – Likely Benign	FOLH1, OR4C13
16	arr 16p11.2(28,312,776-28,980,251)x1	667,474	Loss	Uncertain Significance – Likely Benign	APOB48R, ATP2A1, ATXN2L, CCDC101, CD19, CLN3, EIF3C, EIF3CL, IL27, LAT, LOC390688, LOC440350, NFATC2IP, NUPR1, RABEP2, SH2B1, SPNS1, SULT1A1, SULT1A2, TUFM
17	arr 17q21.31q21.32(41,612,651-42,121,257)x3	508,605	Gain	Uncertain Significance – Likely Benign	ARL17, ARL17P1, KIAA1267, LRRC37A, LRRC37A2, NSF
X	arr Xq26.2(132,937,362-132,974,014)x3	36,651	Gain	Uncertain Significance – Likely Benign	GPC3

Select differentially expressed genes are in bold and underlined; classifications are based on ACMG draft guidelines  
 \*Aberration marked manually and included in report

**Notes:**

- Karyotype Information – no clonal abnormalities were detected.
- Published CNVs (0)

**References:** Werbowetski-Ogilvie, T, Bosse, M, Stewart, M, et al. (2008). Characterization of human embryonic stem cells with features of neoplastic progression. *Nature Biotechnology* 27, 91-97; Wu, H, Kim, K, Mehta, K, et al. (2008). Copy number variant analysis of human embryonic stem cells. *Stem Cells* 26, 1484-1489; Chin, MH, Mason, M, Xie, W, et al. (2009). Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* 5, 111-123; Närvä, E, Autio R, Rahkonen N, et al. (2010). High-resolution DNA analysis of human embryonic stem cell lines reveals culture-induced copy number changes and loss of heterozygosity. *Nature Biotechnology* 28, 371-377

**Recommendations:** For relevant findings, confirmation and localization is recommended. Contact [cytogenetics@wicell.org](mailto:cytogenetics@wicell.org) to request further testing.

**Results Completed By:** ██████████ MS, CG(ASCP)  
**Reviewed and Interpreted By:** ██████████, PhD, FACMG

**aCGH Specifications:**

- Platform: NimbleGen 12x135K array (HG18 WG CGH v3.1 HX12)
- Relative copy number is determined by competitive differential hybridization of labeled genomic DNA to the 135,000 oligonucleotide whole genome tiling array
- Probe length = 60mer, spanning non-repetitive regions of the human genome
- Median probe spacing = 21,500
- Analysis software: NimbleScan™, CGH Fusion (RBS v1.0)™
- Array design, genomic position, genes and chromosome banding are based on HG18.
- Analysis is based on examination of unaveraged and/or 130Kbp (10X) averaged data tracks as noted. Settings for data analysis in Infoquant include an average log-ratio threshold of 0.2, a minimum aberration length of 5 probes, p-value of 0.001. Additional analysis of this data may be performed using different ratio settings and different window averaging to enhance resolution.
- Raw data has not yet been deposited in GEO.
- Reported gains and losses are based on test to reference ratios within CGHfusion™ and the size of aberration.
- Quality assurance monitors: 1) opposite gender reference DNA ratio change in X and Y chromosomes; 2) presence of Xpter and Xq21.3 'pseudoautosomal' (PAR) imbalance; 3) presence of known reference DNA copy number changes. QA measures—PAR (2/2); Reference DNA copy number changes (13); test sample gain or loss of X and Y chromosomes consistent with the opposite gender reference sample.

**Limitations:** This assay will detect aneuploidy, deletions, duplications of represented loci, but will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and insertions), point mutations, loss of heterozygosity (LOH), uniparental disomy or imbalances less than 30kb in size. Copy number variants can be attributable to the test or reference samples used. Exact limits of detectable mosaicism have not been determined, but >20% mosaicism is reported to be visualized by aCGH. Actual chromosomal localization of copy number change is not determined by this assay. Other mapping procedures are required for determining chromosomal localization.

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**Results Transmitted by**  Fax /  Email /  Post  
**Sent By:** \_\_\_\_\_

**Date:** \_\_\_\_\_  
**Sent To:** \_\_\_\_\_

Sponsor: WiCell Research Institute

Accession #: 2010-020961

**Diagnostic Summary Report**

Received: 26 Mar 2010  
 Approved: 01 Apr 2010, 15:52  
 Bill Method: Purchase Order  
 Test Specimen: hES Human

Sample Set	Service (# Tested)	Profile	Assay	Tested	+	+/-	?
#1	Infectious Disease PCR (3)	All Results Negative					

+ = Positive, +/- = Equivocal, ? = Indeterminate

**Service Approvals**

Service	Approved By*	Date
Infectious Disease PCR		01 Apr 2010, 15:52

To assure the SPF status of your research animal colonies, it is essential that you understand the sources, pathobiology, diagnosis and control of pathogens and other adventitious infectious agents that may cause research interference. We have summarized this important information in infectious agent **Technical Sheets**, which you can view by visiting [http://www.criver.com/info/disease\\_sheets](http://www.criver.com/info/disease_sheets).

*\*This report has been electronically signed by laboratory personnel. The name of the individual who approved these results appears in the header of this service report. All services are performed in accordance with and subject to General Terms and Conditions of Sale found in the Charles River Laboratories-Research Models and Services catalogue and on the back of invoices.*



Sponsor: WiCell Research Institute

Accession #: 2010-020961

Product: Not Indicated

Test Specimen: hES Human

Received: 26 Mar 2010

**Molecular Diagnostics Infectious Disease PCR Results Report**

Department Review: Approved by

01 Apr 2010, 15:52\*

**Human Comprehensive Viral PCR Panel**

Sample #: Code :	<u>1</u>	<u>2</u>	<u>3</u>
	WA01-DL-11 5446	WA17-pMCB -02 9243	WA17-pMCB -03 9778
<i>John Cunningham virus</i>	-	-	-
<i>BK virus</i>	-	-	-
<i>Herpesvirus type 6</i>	-	-	-
<i>Herpesvirus type 7</i>	-	-	-
<i>Herpesvirus type 8</i>	-	-	-
<i>Parvovirus B19</i>	-	-	-
<i>Epstein-Barr Virus</i>	-	-	-
<i>Hepatitis A virus</i>	-	-	-
<i>Hepatitis B virus</i>	-	-	-
<i>Hepatitis C virus</i>	-	-	-
<i>HPV-16</i>	-	-	-
<i>HPV-18</i>	-	-	-
<i>Human T-lymphotropic virus</i>	-	-	-
<i>Human cytomegalovirus</i>	-	-	-
<i>HIV-1</i>	-	-	-
<i>HIV-2</i>	-	-	-
<i>Adeno-associated virus</i>	-	-	-
<i>Human Foamy Virus</i>	-	-	-
<i>LCMV PCR</i>	-	-	-
<i>Hantavirus Hantaan PCR</i>	-	-	-
<i>Hantavirus Seoul PCR</i>	-	-	-
<i>Mycoplasma Genus PCR</i>	-	-	-
<i>DNA Spike</i>	PASS	PASS	PASS
<i>RNA Spike</i>	PASS	PASS	PASS
<i>NRC</i>	PASS	PASS	PASS

**Remarks:** - = Negative; I = Inhibition, +/- = Equivocal; + = Positive.

Sample Suitability/Detection of PCR Inhibition:

Sample DNA or RNA is spiked with a low-copy number of a exogenous DNA or RNA template respectively. A spike template-specific PCR assay is used to test for the spike template for the purpose of determining the presence of PCR inhibitors. The RNA spike control is also used to evaluate the reverse-transcription of RNA. Amplification of spike template indicates that there is no detectable inhibition and the assay is valid.

NRC:

The nucleic acid recovery control (NRC) is used to evaluate the recovery of DNA/RNA from the nucleic acid isolation process. The test article is spiked with a low-copy number of DNA/RNA template prior to nucleic acid isolation. A template-specific PCR assay is used to detect the DNA/RNA spike.