Product Information and Testing - Amended

Product Information

Product Name	WA24
Lot Number	WB0074
Parent Material	This material descended from derivation
Depositor	WiCell
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 2 wells of a 6 well plate.
Culture Platform	Feeder Independent Medium: mTeSR1 Matrix: Matrigel
Protocol	WiCell Feeder Independent Protocol
Passage Number	p13 These cells were cultured for 12 passages prior to freeze, 5 of them in mTeSR1/Matrigel. Cells were derived in E8 + PVA on 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	16-November-2010
Vial Label	WB0074 WA24 p13 DF 16NOV10
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	18-MAR -2011

Date of Lot Release	Quality Assurance Approval
18-March-2011	1/3/2014 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: 2021-STR UW HLA#: 64396 Sample Date: 01/03/11
Received Date: 01/03/11

Requestor: WiCell Research Institute
Test Date: 01/04/11 File Name: 110104tcs Report Date: 01/06/11

Sample Name: (label on tube) **2021-STR**

Description: WiCell Research Institute provided genomic DNA
213.66 ug/mL; 260/280 = 1.88

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	9,12
D7S820	6-14	11,12
D13S317	7-15	10,12
D5S818	7-15	11,12
CSF1PO	6-15	11,12
TPOX	6-13	8,11
Amelogenin	NA	X,Y
TH01	5-11	7,9.3
vWA	11, 13-21	15,16

Comments: Based on the 2021-STR DNA dated and received on 01/03/11 from WiCell Research Institute, this sample (UW HLA# 64396) exactly matches the STR profile of the human stem cell line WA24 comprising 16 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA24 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 2021-STR DNA sample submitted corresponds to the WA24 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%.

[redacted] 1/7/11
Date
Molecular Diagnostics Laboratory

[redacted] 1/06/11
Date
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
853980
Page 1 of 1

WiCell Research Institute
[redacted]

December 22, 2010
P.O. #: [redacted]

STERILITY TEST REPORT

Sample Information: hES Cells
1: WA22-WB0064 #5323
2: WA21-WB0070 #2583
3: WA24-WB0074 #7996
4: WA09-WB0072 #5950
5: WA23-WB0073 #3088
6: WA20-WB0071 #7659
7: WA24-WB0066 #8107

Date Received: December 02, 2010
Date in Test: December 07, 2010
Date Completed: December 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	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: Sample(s) WA22-WB0064 # 5323 positive.

[redacted] 12-23-10
QA Reviewer Date

[redacted] 12-23-10
Technical Reviewer Date

Testing conducted in accordance with current Good Manufacturing Practices.

[redacted]

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

APPENDIX

BIONIQUE® TESTING LABORATORIES, INC.

[redacted]

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

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) 63585

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: 1/13/11

Reviewed By [redacted] QA Assistant: [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

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

BIONIQUE TESTING LABORATORIES, INC.
[redacted]

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#: **63585** P.O.# [redacted] DATE REC'D: **12/16/2010**

TEST/CONTROL ARTICLE:

WA24-WB0074 #2021

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0) DATE: **12/16/2010**

INDICATOR CELL LINE (VERO) SEE DNA FLUOROCHROME RECORD SHEET

				DATE
THIOGLYCOLLATE BROTH	DAY 7	+	⊖	**12/23/2010**
	DAY 28	+	⊖	**01/13/2011**
BROTH-FORTIFIED COMMERCIAL				
0.5 mL SAMPLE	DAY 7	+	⊖	**12/23/2010**
6.0 mL BROTH	DAY 28	+	⊖	**01/13/2011**
BROTH-MODIFIED HAYFLICK				
0.5 mL SAMPLE	DAY 7	+	⊖	**12/23/2010**
6.0 mL BROTH	DAY 28	+	⊖	**01/13/2011**
BROTH-HEART INFUSION				
0.5 mL SAMPLE	DAY 7	+	⊖	**12/23/2010**
6.0 mL BROTH	DAY 28	+	⊖	**01/13/2011**

(See Reverse)

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

SAMPLE ID#: **63585**		AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ (-)	+ (-)	12/23/2010
	DAY 14	+ (-)	+ (-)	12/30/2010
	DAY 21	+ (-)	+ (-)	01/06/2011
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ (-)	+ (-)	12/23/2010
	DAY 14	+ (-)	+ (-)	12/30/2010
	DAY 21	+ (-)	+ (-)	01/06/2011
AGAR PLATES-HEART INFUSION	DAY 7	+ (-)	+ (-)	12/23/2010
	DAY 14	+ (-)	+ (-)	12/30/2010
	DAY 21	+ (-)	+ (-)	01/06/2011
BROTH SUBCULTURES (DAY 7)		DATE: **12/23/2010**		
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ (-)	+ (-)	12/30/2010
	DAY 14	+ (-)	+ (-)	01/06/2011
	DAY 21	+ (-)	+ (-)	01/13/2011
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ (-)	+ (-)	12/30/2010
	DAY 14	+ (-)	+ (-)	01/06/2011
	DAY 21	+ (-)	+ (-)	01/13/2011
AGAR PLATES-HEART INFUSION	DAY 7	+ (-)	+ (-)	12/30/2010
	DAY 14	+ (-)	+ (-)	01/06/2011
	DAY 21	+ (-)	+ (-)	01/13/2011

RESULTS: No detectable mycoplasmal contamination

1/13/11
Date

[redacted]
Laboratory Director
[redacted] 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 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.

bionique Testing Laboratories

MYCOPLASMA TESTING SERVICES

BIONIQUE® TESTING LABORATORIES, INC.

[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 # **63585** **M-250** Date Rec'd: **12/16/2010** P.O. # [redacted]

Indicator Cells Inoculated: Date/Initials: 12/16/10 / HS

Fixation: Date/Initials: 12/20/10 / MK

Staining: Date/Initials: 12/20/10 / MK

TEST/CONTROL ARTICLE:

WA24-WB0074 #2021

LOT# **NA**

WiCell QA
WiCell Research Institute

[redacted]

Phone: [redacted]

Fax #:

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: 12/20/10 Results Read by: MK Date of Review: 12/20/10 Reviewed by: SM

WiCell Cytogenetics Report: 003914
WISC 2021

Report Date: December 30, 2010

Case Details:

Cell Line: *WA24-WB0074 (2021)*

Passage #: *14*

Date Completed: *12/30/2010*

Cell Line Gender: *Male*

Investigator: *Dan Felkner*

Specimen: *hESC on Matrigel*

Date of Sample: *12/17/2010*

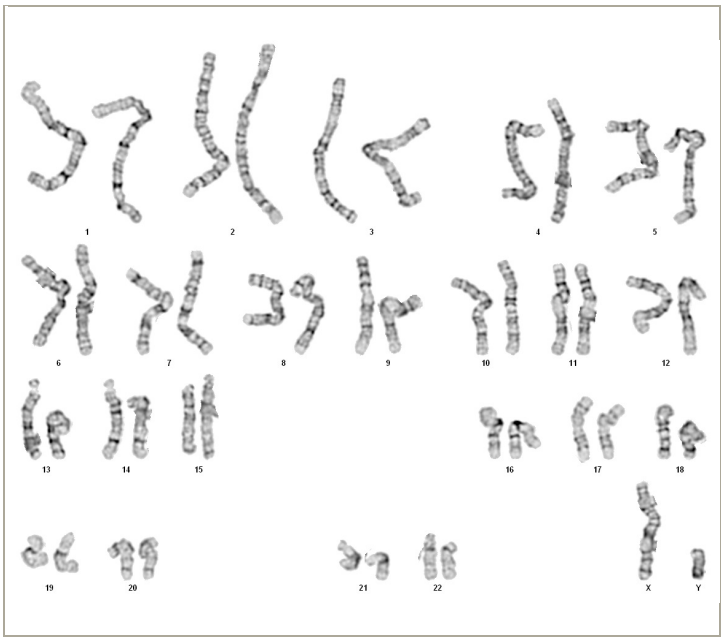
Tests,Reason for: *Lot release testing*

Results: *46,XY*

Completed by [redacted] *MS, CG(ASCP), on 12/29/2010*

Reviewed and interpreted by [redacted] *PhD, FACMG, on 12/30/2010*

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



Cell: *S02-30*

Slide: *3-19 KARYOTYPE*

Slide Type: *Karyotyping*

# of Cells Counted: 20

# of Cells Karyotyped: 4

# of Cells Analyzed: 8

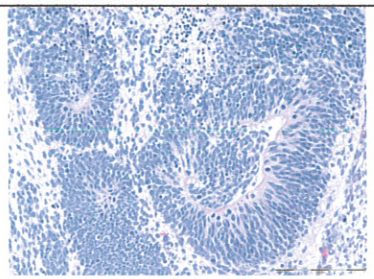
Band Level: 425-600

Results Transmitted by Fax / Email / Post **Date:**_____
Sent By:_____ **Sent To:**_____
QC Review By: _____ **Results Recorded:** _____

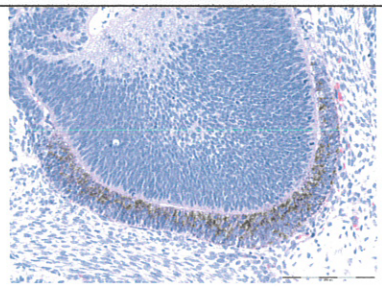
Cell Line: WA24 **Cell Lot Number:** NA **Sample Number:** 5440

ECTODERM

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

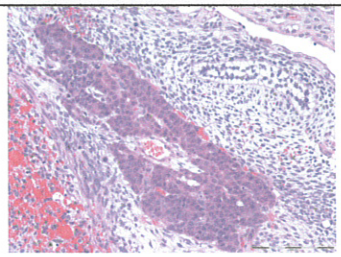


Structure Name: Pigmented Neuro **Magnification:** 200X **Slide ID:** A

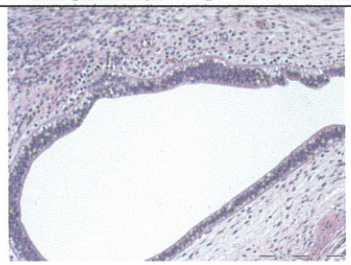


ENDODERM

Structure Name: Hepatoid **Magnification:** 200X **Slide ID:** B

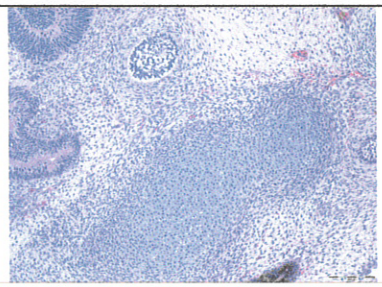


Structure Name: Respiratory **Magnification:** 200x **Slide ID:** B

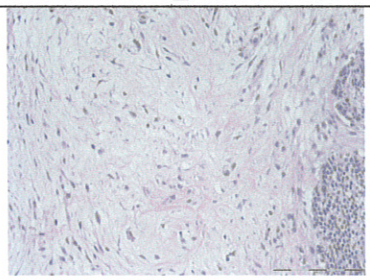


MESODERM

Structure Name: Cartilage **Magnification:** 100X **Slide ID:** A



Structure Name: Myxoid Mesech **Magnification:** 200X **Slide ID:** B



Comments: Structures identified include Ectoderm (2), Mesoderm (2) and Endoderm (3)

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): [redacted]

QA Review (By/Date):

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Date: 12/02/2010 14:51:00

To: WiCell Research Institute ✓

[redacted]

Re: High-resolution HLA results

Patient

Name HLA / MR# received	Dates		HLA DNA-based typing* Method: PCR-SSP A*	B*	C*	Direct Sequencing DRB1*	DRB3*	DRB4*	DRB5*	PCR-SSP DQB1*
WICELL, **7800-HLA**	**DQB SSP**		03:01	07:02g	04:01g	01:03				
64150 /	**A,B,C SSP**	11/29/2010	25:01	35:01g	07:02g	15:01				
11/29/2010	**DRB Seq**	11/29/2010	Class I comment: B*07:02g includes *07:02/61 B*35:01g includes *35:01/42 C*04:01g includes *04:01/09N/30 C*07:02g includes *07:02/50 Class II comment: HLA Allele database: IMGT/A 3.1.0 2010-07-16							

[redacted]

HLA/Molecular Diagnostics Laboratory

12-2-10 1560

Date

[redacted]

HLA/Molecular Diagnostics Laboratory

12/08/10

Date

This test was developed and its performance characteristics determined by the UWHC Clinical Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. However, the FDA does not require licensure of analyte specific reagents since the laboratory is approved, under CLIA, for high complexity testing.

[I:\db\tx\ttmaster\vttmreport.frx]

New York Blood Center
Molecular Analysis Laboratory
[redacted]
Laboratory of Immunohematology
[redacted]

December 9, 2010

WiCell Research Institute
[redacted]

SAMPLE: DNA WA24 7800 (MA#387-10)

Date Received: 11/17/10
Sample Date: 11/17/10

HISTORY: DNA from cell line.

TEST REQUESTED: Genotype for *ABO* and common *RH*

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:* Multiplex PCR-RFLP for *RHD* and *RHCE*C/c*. HEA Beadchip for *RHCE*E/e*.

DNA RESULTS: PCR-RFLP indicated the presence of nt261G, characteristic of O^1 alleles, and nt467T characteristic of A^2 alleles.

Result	Test Method
ABO* A^2O^1	PCR-RFLP
RHD positive for exons 4, 7 and no inactivating pseudogene	Multiplex PCR
RHCE*C/C	Multiplex PCR
RHCE*e/e	HEA 2.1 Assay

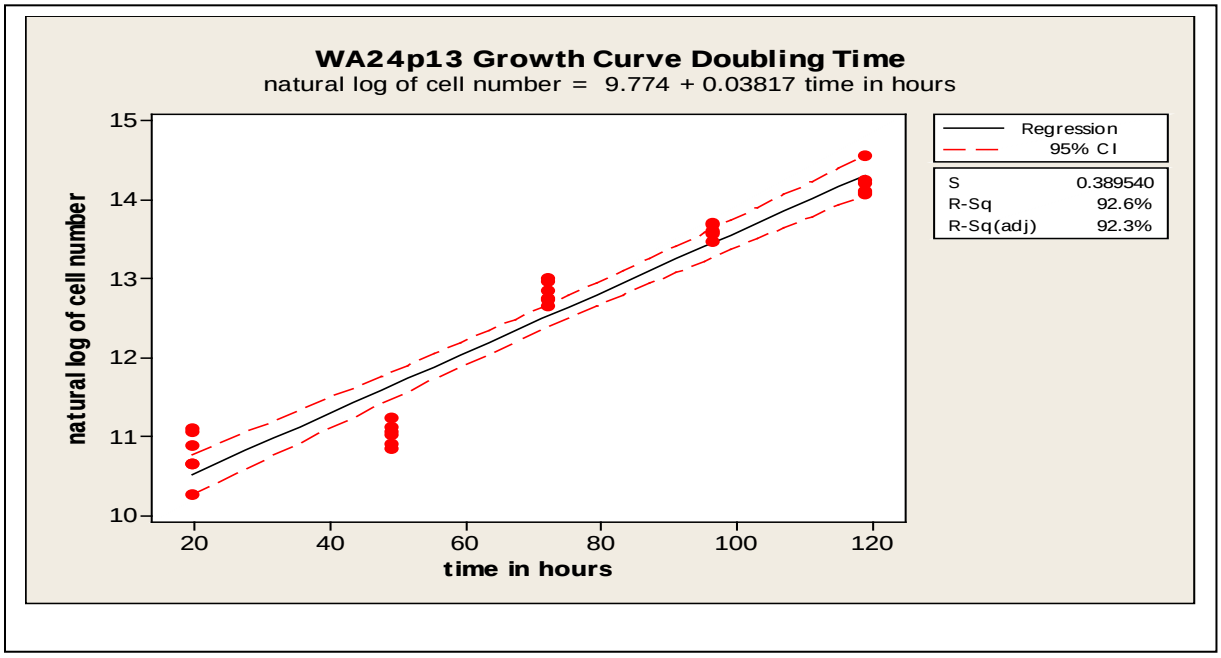
Predicted phenotype: Group A (A2 subgroup) RhD+C+E–c–e+

[redacted]
Manager, Molecular Analysis

[redacted]
Director of Genomics

These *in vitro* diagnostic tests were developed and their performance characteristics established in the Molecular Analysis Laboratory. The tests have not been submitted to the Food and Drug Administration (FDA) for clearance or approval and; therefore, are not FDA-licensed tests. The Molecular Analysis Laboratory is certified under the Clinical Laboratory Improvement Amendment (CLIA) of 1988 as qualified to perform high complexity clinical testing. The New York Blood Center has been approved, by the New York State Department of Health to perform these tests under its current Clinical Laboratory Permit. These results are intended to predict a blood group antigen profile in a patient or donor, and are not intended for clinical diagnosis or as the sole means for patient management decisions. There are situations where testing DNA of a person may not reflect the red cell phenotype and not all performance characteristics have been determined. Nucleotide changes that inactivate gene expression or rare new variant alleles may not be identified in these assays.

Cell Line Information		NSCB QA Use
Sample ID: 9464	Cell lot #: New Derivation	Report reviewed by: JKT
Cell Line: WA24	Report prepared by: MW	Report reviewed on: 14Jan11
Passage: p13	Date cells received: 11-19-10 set	



The regression equation is
natural log of cell number = 9.77 + 0.0382 time in hours

Predictor	Coef	SE Coef	T	P
Constant	9.7737	0.1616	60.49	0.000
time in hours	0.038172	0.002038	18.73	0.000

S = 0.389540 R-Sq = 92.6% R-Sq(adj) = 92.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	53.254	53.254	350.95	0.000
Residual Error	28	4.249	0.152		
Total	29	57.50			

Slope ± 95% C.I. 0.038 ± 0.004

Doubling Time ± 95% C.I.

19.4 hours ± 2.2 hours

21.6 hours – 17.2 hours

Cell Line Information		NSCB QA Use
Sample ID: 9464	Cell lot #: New Derivation	Report reviewed by: JKT
Cell Line: WA24	Report prepared by: MW	Report reviewed on: 14Jan11
Passage: p13	Date cells received: 11-19-10 set	

Photo Day 0- Colonies before splitting

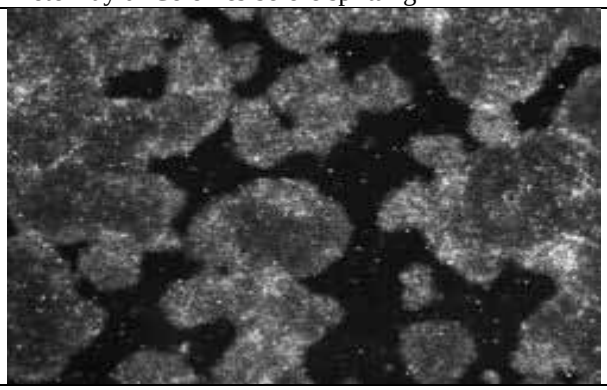


Photo Day 1

Photo Day 2

Photo Day 3

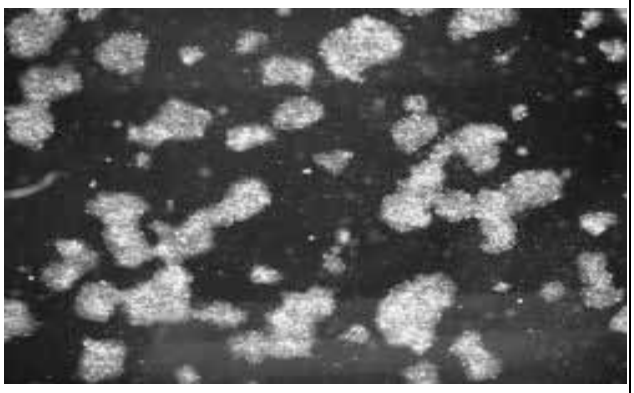


Photo Day 4

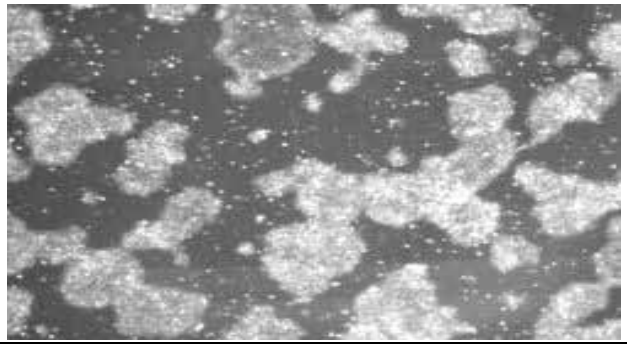
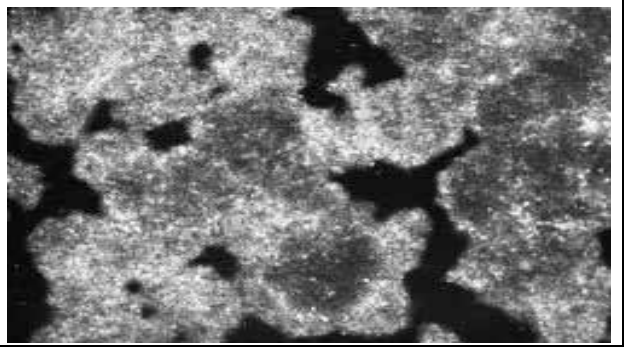


Photo Day 5

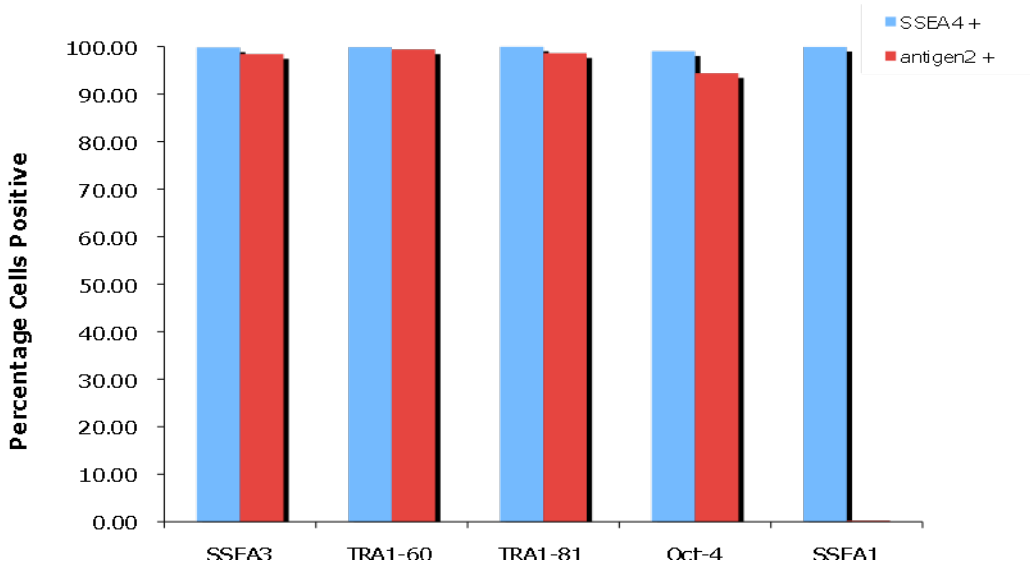


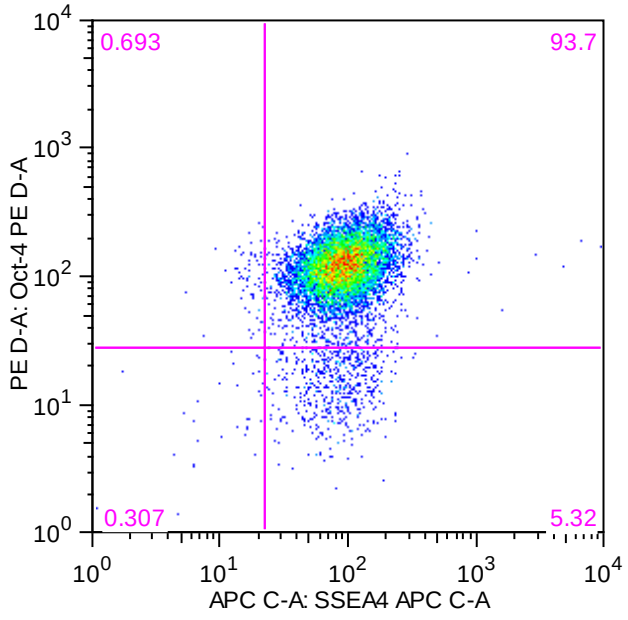
Procedures performed:
SOP-CH-101 SOP-CH-102
SOP-CH-103 SOP-CH-105

Cell Line: WA24 TeSR/MG
Passage 12
Sample ID: 2471-FAC

Date of: *(mm/dd/yy)*
acquisition: 11/16/10
file creation: 11/19/10
file submission: 11/19/10

antigen2:	SSEA4 - antigen2 +	SSEA4 + antigen2 +	SSEA4 + antigen2 -	SSEA4 - antigen2 -	ALL SSEA4 +	ALL antigen2 +
SSEA3	0.12	98.30	1.52	0.04	99.82	98.42
TRA1-60	0.11	99.30	0.57	0.00	99.87	99.41
TRA1-81	0.00	98.60	1.42	0.00	100.02	98.60
Oct-4	0.69	93.70	5.32	0.31	99.02	94.39
SSEA1	0.00	0.25	99.70	0.09	99.95	0.25





CD29-
2471_Test.fcs
Event Count: 10103

WiCell Cytogenetics Report: 003812 WISC3577

Report Date: *7/5/2011*
Date of Sample: *11/2/2010*
Investigator: [redacted]
Reason for Testing: *New Derivation*
Specimen: *hESC on Matrigel, Albumin Free Medium*
Karyotype Results: *46,XY*

Test: *WA24p9 (Male)*
Reference: *WA09-MCB-01-E.3p19(2) (Female)*
Project: [redacted]
Funding: [redacted]
CGH Accession #: *000399*
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 **41** copy number gains and losses identified, including **2** pseudoautosomal regions and **11** copy number changes due to the reference DNA*
- *There is a >1Mb gain at 10q11.22. This CNV is likely cell line specific, is in a region of known copy number variation, and likely a benign finding.*
- *Select CNVs are detailed in the table below*

Chr	Band (Genomic Position)	Width	Aberration Type	Classification	Genes
1	arr 1p36.21(12,893,486-13,498,981)x1	605,494	Loss	Uncertain Significance – Likely Benign	LOC653606, PRAMEF14, PRAMEF16, PRAMEF18, PRAMEF19, PRAMEF20, PRAMEF21, PRAMEF3, PRAMEF5, PRAMEF6, PRAMEF7, PRAMEF8, PRAMEF9
1	arr 1p36.11(25,458,059-25,616,508)x3	158,449	Gain	Uncertain Significance – Likely Benign	RHCE, RHD, TMEM50A
3	arr 3p14.1(65,160,247-65,209,465)x3	49,217	Gain	Uncertain Significance – Likely Benign	
5	arr 5p15.33(780,726-846,031)x1	65,304	Loss	Uncertain Significance – Likely Benign	ZDHHC11
7	arr 7q22.1(101,904,922-102,258,666)x3	353,744	Gain	Uncertain Significance – Likely Benign	FBXL13, LRWD1, MGC119295, POLR2J, POLR2J2, POLR2J3, RASA4
7	arr 7q34(141,322,586-141,449,630)x3	127,044	Gain	Uncertain Significance – Likely Benign	MGAM, TAS2R38
7	arr 7q35(143,528,320-143,705,123)x1	176,803	Loss	Uncertain Significance – Likely Benign	**ARHGEF5**, FLJ43692, OR2A1, OR2A42, OR2A7
8	arr 8p21.3(21,462,240-21,584,539)x3	122,299	Gain	Uncertain Significance – No Sub-Classification	GFRA2
10	arr 10p11.21(37,441,219-37,524,112)x3	82,893	Gain	Uncertain Significance – Likely Benign	ANKRD30A
10	arr 10q11.22(46,125,141-47,228,866)x3	1,103,725	Gain	Uncertain Significance – Likely Benign	ANXA8, ANXA8L1, ANXA8L2, GPRIN2, PPYR1, SYT15
10	arr 10q11.22(47,725,967-47,901,718)x3	175,750	Gain	Uncertain Significance – Likely Benign	ANXA8, ANXA8L1
10	arr 10q11.22(48,555,198-48,954,528)x3	399,330	Gain	Uncertain Significance – Likely Benign	
12	arr 12q24.33(130,301,788-130,490,188)x1	188,400	Loss	Uncertain Significance – Likely Benign	
19	arr 19q13.32q13.33(53,686,843-53,851,993)x1	165,149	Loss	Uncertain Significance – No Sub-Classification	CA11, DBP, FAM83E, LMTK3, LOC126147, RPL18, SPACA4, SPHK2, SULT2B1
22	arr 22q11.23(23,992,673-24,259,370)x1	266,696	Loss	Uncertain Significance – Likely Benign	IGLL3, LRP5L
*X	arr Xq26.2(132,944,488-132,974,884)x3	30,395	Gain	Uncertain Significance – Likely Benign	GPC3
X	arr Xq28(153,064,834-153,165,618)x1	100,784	Loss	Uncertain Significance – Likely Benign	OPN1LW, OPN1MW, OPN1MW2, TEX28

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 abnormalities were detected at the stated band level of resolution*
- *Published CNVs (1) – Narva et al: arr 10q11.22(46,125,141-47,228,866)x3*

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 to request further testing.*

Results Completed By: [redacted]
Reviewed and Interpreted By: [redacted] *PhD, FACMG*

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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 (11); 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.*

Results Transmitted by ☐ Fax / ☐ Email / ☐ Post **Date:**_____
Sent By:_____ **Sent To:**_____

Charles River Research Animal Diagnostic Services

[redacted]

Sponsor: WiCell Research Institute **Accession #: 2010-048114**

Diagnostic Summary Report

[redacted]

Received: 16 Nov 2010
Approved: 18 Nov 2010, 09:30

Bill Method: PO# [redacted]
Test Specimen: 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	[redacted]	18 Nov 2010, 09:27

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.

**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.*

Charles River Research Animal Diagnostic Services
[redacted]

Sponsor: WiCell Research Institute **Accession #: 2010-048114**

Product: Not Indicated **Test Specimen:** Human **Received:** 16 Nov 2010

Molecular Diagnostics Infectious Disease PCR Results Report

Department Review: Approved by [redacted] 18 Nov 2010, 09:27*

Human Comprehensive Virus Panel

Sample #: / Code :	1 WA22-WB0046 #5128	2 WA23-WB0067 #5010	3 WA24-WB0066 #9532
John Cunningham virus	-	-	-
BK virus	-	-	-
Herpesvirus type 6	-	-	-
Herpesvirus type 7	-	-	-
Herpesvirus type 8	-	-	-
Parvovirus B19	-	-	-
Epstein-Barr Virus	-	-	-
Hepatitis A virus	-	-	-
Hepatitis B virus	-	-	-
Hepatitis C virus	-	-	-
HPV-16	-	-	-
HPV-18	-	-	-
Human T-lymphotropic virus	-	-	-
Human cytomegalovirus	-	-	-
HIV-1	-	-	-
HIV-2	-	-	-
Adeno-associated virus	-	-	-
Human Foamy Virus	-	-	-
LCMV PCR	-	-	-
Hantavirus Hantaan PCR	-	-	-
Hantavirus Seoul PCR	-	-	-
Mycoplasma Genus PCR	-	-	-
DNA Spike	**PASS**	**PASS**	**PASS**
RNA Spike	**PASS**	**PASS**	**PASS**
NRC	**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.

**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.*