



## Product Information and Testing - Amended

### Product Information

Product Name	WA21
Lot Number	WB0089
Parent Material	WA21-WB0051
Depositor	WiCell
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 2 wells of a 6 well plate.
Culture Platform	Feeder Free
	Medium: Conditioned Medium
	Matrix: Matrigel
Protocol	WiCell Feeder Free Protocol
Passage Number	p24  These cells were cultured for 23 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	20-June-2011
Vial Label	WB0089 WA21 P24 JB 20JUNE11
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
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. General Cell Line Testing CoA added to lot CoA.	26-JUL-2013
Original CoA	19-SEPT-2011

Date of Lot Release	Quality Assurance Approval
19-September-2011	<p style="text-align: right;">1/3/2014</p> <p style="text-align: center;"><b>X</b> AMC</p> <p>AMC Quality Assurance Signed by: <span style="background-color: black; color: black;">XXXXXXXXXX</span></p>



## Short Tandem Repeat Analysis\*

Sample Report: 10174-STR

UW HLA#: 65833

Sample Date: 08/12/11

Lab Received 08/12/11

Requestor: WiCell Research Institute

Test Date: 08/16/11

File Name: 110817blb

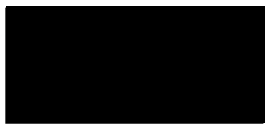
Report Date: 08/17/11

Sample Name: (label on tube) 10174-STR

Description: WI Cell Research Institute provided  
genomic DNA  
256.74 ug/mL 260/280=1.96

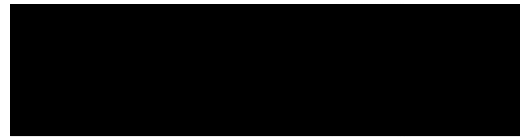
Locus	Repeat #	STR Genotype
D16S539	5, 8-15	11,11
D7S820	6-14	9,9
D13S317	7-15	11,12
D5S818	7-15	11,11
CSF1PO	6-15	12,13
TPOX	6-13	8,12
Amelogenin	NA	X,Y
TH01	5-11	8,9
vWA	11, 13-21	18,18

**Comments:** Based on the 10174-STR DNA dated and received on 08/12/11 from WiCell, this sample (UW HLA# 65833) exactly matches the STR profile of the human stem cell line WA21, comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA21 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 10174-STR DNA sample submitted corresponds to the WA21 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%.



  
 \_\_\_\_\_  
 Manager Date

Molecular Diagnostics Laboratory



  
 \_\_\_\_\_  
 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.

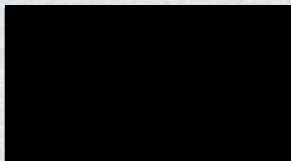
Test Facility:



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  
874340  
Page 1 of 1

WiCell Research Institute



August 23, 2011  
P.O. #:

### STERILITY TEST REPORT

**Sample Information:** hES Cells  
1: MEF SOP-CC-006D34 10179 (2)  
2: WA21-WB0089 10180 (2)  
3: WA21-WB0096 10181 (2)  
4: WA01-WB0097 10182 (2)  
5: WA24-WB0068 10185 (2)

**Date Received:** August 03, 2011  
**Date in Test:** August 08, 2011  
**Date Completed:** August 22, 2011

**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	10	10
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
<b>RESULTS</b>	8 NEGATIVE 2 POSITIVE	9 NEGATIVE 1 POSITIVE

Note: The SCD samples for MEF SOP-CC-006D34 10179 and WA01-WB0097 10182 were positive.

The FTM sample for MEF SOP-CC-006D34 10179 was positive.



QA Reviewer

08-24-11

Date



Technical Reviewer

08-24-11

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 - G M P**

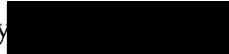
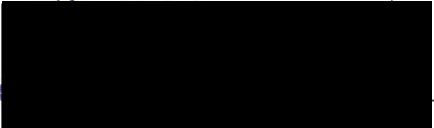
<u>TEST PERFORMED</u>	<u>PROCEDURAL REFERENCE</u>	<u>TEST PERFORMED</u>	<u>PROCEDURAL REFERENCE</u>
<input checked="" type="checkbox"/> M-250	SOP's 3008, 3011, 3013	<input type="checkbox"/> M-700	SOP's 3008, 3009, 3010
<input type="checkbox"/> M-300	SOP's 3008, 3014	<input type="checkbox"/> M-800	SOP's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

Bionique Sample ID #(s) 66260

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: 8/31/11

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.

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

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## 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

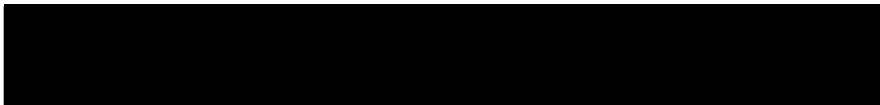
Page 1 of 2

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#: **66260** P.O.#:  DATE REC'D: **08/02/2011**

TEST/CONTROL ARTICLE:

**WA21-WB0089 10174**

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0)

DATE: **08/03/2011**

INDICATOR CELL LINE (VERO)

SEE DNA FLUOROCHROME RECORD SHEET

DATE

THIOGLYCOLLATE BROTH	DAY 7	+	⊖	<b><u>08/10/2011</u></b>
	DAY 28	+	⊖	<b><u>08/31/2011</u></b>
BROTH-FORTIFIED COMMERCIAL				
<b><u>0.5</u></b> mL SAMPLE	DAY 7	+	⊖	<b><u>08/10/2011</u></b>
<b><u>6.0</u></b> mL BROTH	DAY 28	+	⊖	<b><u>08/31/2011</u></b>
BROTH-MODIFIED HAYFLICK				
<b><u>0.5</u></b> mL SAMPLE	DAY 7	+	⊖	<b><u>08/10/2011</u></b>
<b><u>6.0</u></b> mL BROTH	DAY 28	+	⊖	<b><u>08/31/2011</u></b>
BROTH-HEART INFUSION				
<b><u>0.5</u></b> mL SAMPLE	DAY 7	+	⊖	<b><u>08/10/2011</u></b>
<b><u>6.0</u></b> mL BROTH	DAY 28	+	⊖	<b><u>08/31/2011</u></b>


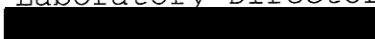
(See Reverse)

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

SAMPLE ID#: <b>66260</b>		AEROBIC	MICROAEROPHILIC	DATE
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>08/10/2011</u>
	DAY 14	+ ⊖	+ ⊖	<u>08/17/2011</u>
	DAY 21	+ ⊖	+ ⊖	<u>08/24/2011</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>08/10/2011</u>
	DAY 14	+ ⊖	+ ⊖	<u>08/17/2011</u>
	DAY 21	+ ⊖	+ ⊖	<u>08/24/2011</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>08/10/2011</u>
	DAY 14	+ ⊖	+ ⊖	<u>08/17/2011</u>
	DAY 21	+ ⊖	+ ⊖	<u>08/24/2011</u>
<b><u>BROTH SUBCULTURES (DAY 7)</u></b>		DATE: <u>08/10/2011</u>		
AGAR PLATES-FORTIFIED COMMERCIAL	DAY 7	+ ⊖	+ ⊖	<u>08/17/2011</u>
	DAY 14	+ ⊖	+ ⊖	<u>08/24/2011</u>
	DAY 21	+ ⊖	+ ⊖	<u>08/31/2011</u>
AGAR PLATES-MODIFIED HAYFLICK	DAY 7	+ ⊖	+ ⊖	<u>08/17/2011</u>
	DAY 14	+ ⊖	+ ⊖	<u>08/24/2011</u>
	DAY 21	+ ⊖	+ ⊖	<u>08/31/2011</u>
AGAR PLATES-HEART INFUSION	DAY 7	+ ⊖	+ ⊖	<u>08/17/2011</u>
	DAY 14	+ ⊖	+ ⊖	<u>08/24/2011</u>
	DAY 21	+ ⊖	+ ⊖	<u>08/31/2011</u>

RESULTS: No detectable mycoplasmal contamination

8/31/11  
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 # 66260                      M-250                      Date Rec'd: 08/02/2011                      P.O. #

Indicator Cells Inoculated:                      Date/Initials:                      8/4/11 / nk

Fixation:                      Date/Initials:                      8/8/11 / nk

Staining:                      Date/Initials:                      8/8/11 / nk

TEST/CONTROL ARTICLE:

WA21-WB0089 10174

LOT# NA

WiCell QA  
WiCell Research Institute



Phone:

Fax #:

**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: 8/8/11                      Results Read by: nk                      Date of Review: 8/8/11                      Reviewed by: SM

**REVISED**

**Report Date: September 14, 2011**    **Revised to reflect that cell line gender is male**

**Cell Line: WA21-WB0089 10174**

**Cell Line Gender: Female**

**Passage #: 26**

**Revised--Cell Line Gender: Male**

**Date of Sample: 8/3/2011**

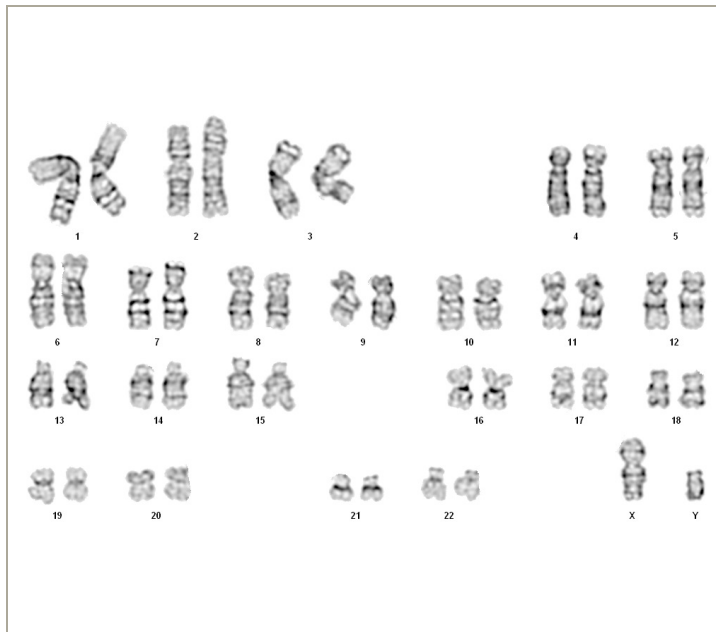
**Reason for Testing: Lot Release Testing**

**Date Completed: 8/10/2011**

**Investigator: ██████████ Wisconsin  
International Stem Cell Bank**

**Specimen: hESC on Matrigel**

**Results: 46,XY**



**Cell: S02-22**

**Slide: 2-R1 (13) KARYOYPE**

**Slide Type: Karyotyping**

**# of Cells Counted: 20**

**# of Cells Karyotyped: 4**

**# of Cells Analyzed: 8**

**Band Level: 400-550**

**Interpretation:**

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

**Please note: this karyotype reports an XY complement in all cells examined; the test requisition form accompanying this specimen indicates a female cell line. [Revised by ██████████ with information from ██████████ that cell line is male]**

**Completed by ██████████ MS, CG(ASCP), on 8/10/2011**

**Reviewed and interpreted by ██████████, PhD, FACMG, on 8/10/2011**

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

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

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

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

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

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