# Product Information and Testing - Amended

## Product Information

| | |
|---|---|
| Product Name | WA20 |
| Lot Number | WB0071 |
| Depositor | WiCell |
| Banked by | WiCell |
| Thaw Recommendation | Thaw 1 vial into 1 well of a 6 well plate. |
| Culture Platform | Feeder Free |
| | Medium: Conditioned Medium |
| | Matrix: Matrigel |
| Protocol | WiCell Feeder Free Protocol |
| Passage Number | p21<br><br>These cells were cultured for 20 passages prior to freeze. WiCelladds +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 | 05-November-2010 |
| Vial Label | WB0071<br>WA20<br>p21 MW<br>05NOV10 |
| 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.<br>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<br>SOP-CH-214 |
| HLA | UW Molecular Diagnostics Laboratory | PowerPlex 1.2 System by Promega |
| ABO | American Red Cross | For ABO:<br>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.<br>For RHD:<br>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<br>SOP-CH-102<br>SOP-CH-103<br>SOP-CH-105 |
| Array Comparative Genomic Hybridization (aCGH) | WiCell | SOP-CH-308<br>SOP-CH-309<br>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 | 18-MAR-2011 |

| Date of Lot Release | Quality Assurance Approval |
|---|---|
| 18-March-2011 | 1/3/2014<br>X AMC<br>AMC<br>Quality Assurance<br>Signed by: [redacted] |

UW Health
University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

# Short Tandem Repeat Analysis*

Sample Report: 2209-STR | UW HLA#: 64438 | Sample Date: 01/07/11
Received Date: 01/07/11

Requestor: WiCell Research Institute

Test Date: 01/11/11 | File Name: 110112cln | Report Date: 01/14/11

Sample Name: (label on tube) 2209-STR

Description: DNA Extracted by WiCell
191.7 ng/µL; 260/280 = 1.92

| Locus | Repeat # | STR Genotype |
|---|---|---|
| D16S539 | 5, 8-15 | 9,11 |
| D7S820 | 6-14 | 11,12 |
| D13S317 | 7-15 | 13,14 |
| D5S818 | 7-15 | 11,12 |
| CSF1PO | 6-15 | 11,14 |
| TPOX | 6-13 | 8,11 |
| Amelogenin | NA | X,X |
| TH01 | 5-11 | 6,9 |
| vWA | 11, 13-21 | 16,20 |

**Comments: Based on the 2209-STR DNA dated and received on 01/07/11 from WI Cell, this sample (UW HLA# 64438) exactly matches the STR profile of the human stem cell line WA20 comprising 16 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA20 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 2209-STR DNA sample submitted corresponds to the WA20 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] 1/14/11
Date
Molecular Diagnostics Laboratory

[redacted] 01/14/11
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<br>2 POSITIVE | 12 NEGATIVE<br>2 POSITIVE |

Note: Sample(s) WA22-WB0064 # 5323 positive.

[redacted]
QA Reviewer 12-23-10
Date

[redacted]
Technical Reviewer 12-23-10
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) 63723 63724

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: 2/1/11 | 2/2/11

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

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MYCOPLASMA TESTING SERVICES

BIONIQUE TESTING LABORATORIES, INC.
[redacted]



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#: **63724** P.O.#: [redacted] DATE REC'D: **01/04/2011**

TEST/CONTROL ARTICLE:

**WA20-WB0071 #2209**

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0) DATE: **01/05/2011**

INDICATOR CELL LINE (VERO) SEE DNA FLUOROCHROME RECORD SHEET

| | | | DATE |
|---|---|---|---|
| THIOGLYCOLLATE BROTH | DAY 7 | + ⊖ | 01/12/2011 |
| | DAY 28 | + ⊖ | 02/02/2011 |
| BROTH-FORTIFIED COMMERCIAL | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 01/12/2011 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 02/02/2011 |
| BROTH-MODIFIED HAYFLICK | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 01/12/2011 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 02/02/2011 |
| BROTH-HEART INFUSION | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 01/12/2011 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 02/02/2011 |

(See Reverse)

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

| SAMPLE ID#: **63724** | | AEROBIC | MICROAEROPHILIC | DATE |
|---|---|---|---|---|
| AGAR PLATES-FORTIFIED COMMERCIAL | DAY 7 | + ⊖ | + ⊖ | 01/12/2011 |
| | DAY 14 | + ⊖ | + ⊖ | 01/19/2011 |
| | DAY 21 | + ⊖ | + ⊖ | 01/26/2011 |
| AGAR PLATES-MODIFIED HAYFLICK | DAY 7 | + ⊖ | + ⊖ | 01/12/2011 |
| | DAY 14 | + ⊖ | + ⊖ | 01/19/2011 |
| | DAY 21 | + ⊖ | + ⊖ | 01/26/2011 |
| AGAR PLATES-HEART INFUSION | DAY 7 | + ⊖ | + ⊖ | 01/12/2011 |
| | DAY 14 | + ⊖ | + ⊖ | 01/19/2011 |
| | DAY 21 | + ⊖ | + ⊖ | 01/26/2011 |
| **BROTH SUBCULTURES (DAY 7)** | | DATE: **01/12/2011** | | |
| AGAR PLATES-FORTIFIED COMMERCIAL | DAY 7 | + ⊖ | + ⊖ | 01/19/2011 |
| | DAY 14 | + ⊖ | + ⊖ | 01/26/2011 |
| | DAY 21 | + ⊖ | + ⊖ | 02/02/2011 |
| AGAR PLATES-MODIFIED HAYFLICK | DAY 7 | + ⊖ | + ⊖ | 01/19/2011 |
| | DAY 14 | + ⊖ | + ⊖ | 01/26/2011 |
| | DAY 21 | + ⊖ | + ⊖ | 02/02/2011 |
| AGAR PLATES-HEART INFUSION | DAY 7 | + ⊖ | + ⊖ | 01/19/2011 |
| | DAY 14 | + ⊖ | + ⊖ | 01/26/2011 |
| | DAY 21 | + ⊖ | + ⊖ | 02/02/2011 |

RESULTS: No detectable mycoplasmal contamination

2/2/11
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.

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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 # **63724** **M-250** Date Rec'd: **01/04/2011** P.O. # [redacted]

Indicator Cells Inoculated: Date/Initials: 1/6/11 / MK

Fixation: Date/Initials: 1/10/11 / MK

Staining: Date/Initials: 1/10/11 / MK

TEST/CONTROL ARTICLE:

**WA20-WB0071 #2209**

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: 1/10/11 Results Read by: MK Date of Review: 1/10/11 Reviewed by: SD

# WiCell Cytogenetics Report: 003934
## WISC 2209

**Report Date:** January 10, 2011

## Case Details:

***Cell Line:*** *WA20-WB0071 2209*

***Passage #:*** *24*

***Date Completed:*** *1/10/2011*

***Cell Line Gender:*** *Female*

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

***Specimen:*** *hESC on Matrigel*

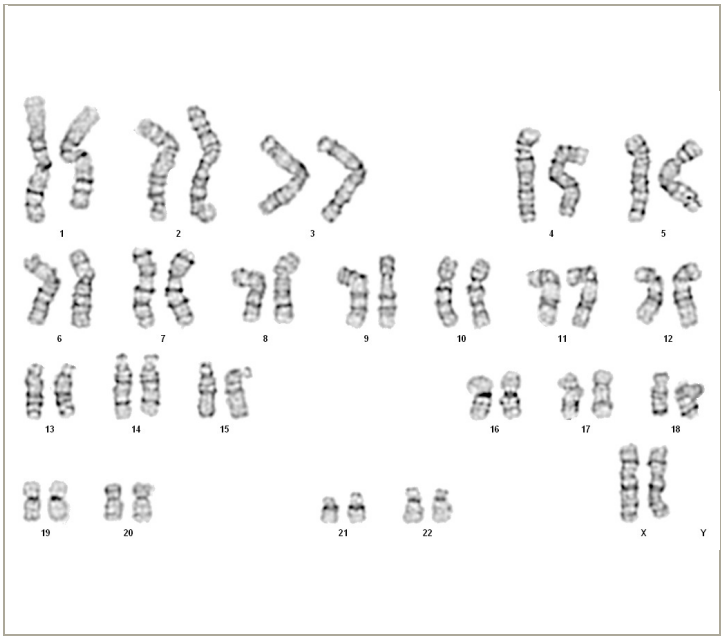
***Date of Sample:*** *1/3/2011*

***Tests,Reason for:*** *lot release testing*

***Results:*** *46,XX*
*Completed by* [redacted] *CG(ASCP), on 1/10/2011*

*Reviewed and interpreted by* [redacted] *PhD, FACMG, on 1/10/2011*

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



***Cell:*** *S01-19*

***Slide:*** *2(8)KARYOTYPE*

***Slide Type:*** *Karyotyping*

***# of Cells Counted: 20***

***# of Cells Karyotyped: 4***

***# of Cells Analyzed: 8***

***Band Level: 400-500***

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