# Thaw and Culture Details

| | |
|---|---|
| Cell Line Name | **ES03** |
| WiCell Lot Number | **DL-03** |
| Parent Material | ES03-MCB-01 |
| Provider | ESI/Biotime |
| Banked by | WiCell |
| Thaw and Culture Recommendations | Thaw 1 vial into 1 well of a 6 well plate. |
| Culture Platform | Feeder Dependent |
| | Medium: hES Medium |
| | Matrix: MEF |
| Protocol | WiCell Feeder Dependent Protocol |
| Passage Number | p67<br>These cells were cultured for 66 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 Vialed | 19-June-2009 |
| Vial Label | ES03-DL-03<br>P67 LD<br>19 JUNE 09<br>SOPCC035D |
| 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. |

## *Testing Performed by WiCell*

| Test Description | Test Provider | Test Method | Test Specification | Result |
|---|---|---|---|---|
| Karyotype by G-banding | WiCell | SOP-CH-003 | Expected karyotype | See Report |
| Post-Thaw Viable Cell Recovery | WiCell | SOP-CH-305 | ≥ 15 Undifferentiated Colonies prior to passage,<br>≤ 30% Differentiation prior to passage, and recoverable attachment after passage | Pass |
| Identity by STR | UW Molecular Diagnostics Laboratory | PowerPlex 1.2 System by Promega | Consistent with STR profile of deposited cell line | Pass |
| Sterility - Direct transfer method | Apptec | 30744 | No contamination detected | Pass |
| Mycoplasma | Bionique | M250 | No contamination detected | Pass |
| 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 | Report - no specification | See report |

| Date of Lot Release | Quality Assurance Approval |
| --- | --- |
| 12-November-2009 | 5/27/2020<br>X HEB<br>HEB<br>Quality Assurance<br>Signed by: Bruner, Haley |

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

# Short Tandem Repeat Analysis*

**Sample Report: 9440-STR** UW HLA#: 61564 Sample Date: 08/25/09
Received Date: 08/25/09

Requestor: WiCell Research Institute
Test Date: 09/04/09 File Name: 090905 Report Date: 09/11/09

**Sample Name:** (label on tube) **9440-STR** **Description:** DNA Extracted by WiCell

247.62 ug/mL; 260/280 = 1.86

| Locus | Repeat # | STR Genotype |
|---|---|---|
| D16S539 | 5, 8-15 | 9,9 |
| D7S820 | 6-14 | 11,12 |
| D13S317 | 7-15 | 9,12 |
| D5S818 | 7-15 | 10,12 |
| CSF1PO | 6-15 | 12,12 |
| TPOX | 6-13 | 8,8 |
| Amelogenin | NA | X,X |
| TH01 | 5-11 | 7,9 |
| vWA | 11, 13-21 | 17,18 |

**Comments: Based on the DNA 9440-STR dated and received on 08/25/09 from WI Cell, this sample (UW HLA# 61564) matches exactly the STR profile of the human stem cell line ES03 comprising 13 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human ES03 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 9440-STR DNA sample submitted corresponds to the ES03 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] 9/10/09
anager Date
HLA/Molecular Diagnostics Laboratory

[redacted] 09/10/09
tor 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.

File: Final STR Report

Test Facility:
1265 Kennestone Circle
Marietta, GA 30066



WuXi AppTec



WiCell Research Institute
[redacted]

September 23, 2009
P.O. #: [redacted]

# STERILITY TEST REPORT

**Sample Information:** hES Cells
1: WA09-DL-10 NSCB #1524
2: ES03-DL-3 NSCB # 9440

**Date Received:** September 03, 2009
**Date in Test:** September 04, 2009
**Date Completed:** September 18, 2009

**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** | 4 | 4 |
| **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** | 4 NEGATIVE | 4 NEGATIVE |

[redacted] 09-23-09
QA Reviewer Date

[redacted] 09-23-09
Technical Reviewer Date

*Testing conducted in accordance with current Good Manufacturing Practices.*

Mycoplasma Testing Services
Safe Cells

BIONIQUE TESTING LABORATORIES, INC.
156 FAY BROOK DRIVE
SARANAC LAKE, NY 12983
PHONE: 518-891-2356 FAX: 518-891-5753



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#: **58232** P.O.#: [redacted] DATE REC'D: **08/04/2009**

TEST/CONTROL ARTICLE:

**ES03-DL-3 #9440**

LOT#: **NA**

DIRECT CULTURE SET-UP (DAY 0) DATE: **08/05/2009**

INDICATOR CELL LINE (VERO) SEE DNA FLUOROCHROME RECORD SHEET

| | | | DATE |
|---|---|---|---|
| THIOGLYCOLLATE BROTH | DAY 7 | + ⊖ | 08/12/2009 |
| | DAY 28 | + ⊖ | 09/02/2009 |
| BROTH-FORTIFIED COMMERCIAL | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 08/12/2009 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 09/02/2009 |
| BROTH-MODIFIED HAYFLICK | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 08/12/2009 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 09/02/2009 |
| BROTH-HEART INFUSION | | | |
| 0.5 mL SAMPLE | DAY 7 | + ⊖ | 08/12/2009 |
| 6.0 mL BROTH | DAY 28 | + ⊖ | 09/02/2009 |

(See Reverse)

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

| SAMPLE ID#: 58232 | | AEROBIC | MICROAEROPHILIC | DATE |
|---|---|---|---|---|
| AGAR PLATES-FORTIFIED COMMERCIAL | DAY 7 | + ⊖ | + ⊖ | 08/12/2009 |
| | DAY 14 | + ⊖ | + ⊖ | 08/19/2009 |
| | DAY 21 | + ⊖ | + ⊖ | 08/26/2009 |
| AGAR PLATES-MODIFIED HAYFLICK | DAY 7 | + ⊖ | + ⊖ | 08/12/2009 |
| | DAY 14 | + ⊖ | + ⊖ | 08/19/2009 |
| | DAY 21 | + ⊖ | + ⊖ | 08/26/2009 |
| AGAR PLATES-HEART INFUSION | DAY 7 | + ⊖ | + ⊖ | 08/12/2009 |
| | DAY 14 | + ⊖ | + ⊖ | 08/19/2009 |
| | DAY 21 | + ⊖ | + ⊖ | 08/26/2009 |
| **BROTH SUBCULTURES (DAY 7)** | | DATE: 08/12/2009 | | |
| AGAR PLATES-FORTIFIED COMMERCIAL | DAY 7 | + ⊖ | + ⊖ | 08/19/2009 |
| | DAY 14 | + ⊖ | + ⊖ | 08/26/2009 |
| | DAY 21 | + ⊖ | + ⊖ | 09/02/2009 |
| AGAR PLATES-MODIFIED HAYFLICK | DAY 7 | + ⊖ | + ⊖ | 08/19/2009 |
| | DAY 14 | + ⊖ | + ⊖ | 08/26/2009 |
| | DAY 21 | + ⊖ | + ⊖ | 09/02/2009 |
| AGAR PLATES-HEART INFUSION | DAY 7 | + ⊖ | + ⊖ | 08/19/2009 |
| | DAY 14 | + ⊖ | + ⊖ | 08/26/2009 |
| | DAY 21 | + ⊖ | + ⊖ | 09/02/2009 |

RESULTS: No detectable mycoplasmal contamination

9/2/09
Date

[redacted]

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.

Mycoplasma Testing Services
Safe Cells

MYCOPLASMA
TESTING SERVICES

BIONIQUE TESTING LABORATORIES, INC
156 Fay Brook Drive
Saranac Lake, NY 12983
Phone: 518-891-2356 FAX: 518-891-5753

APPENDIX I

| | |
|---|---|
| Document #: | DCF3008A |
| Edition #: | 06 |
| Effective date: | 9/17/2003 |
| Title: | DNA FLUOROCHROME ASSAY RESULTS |

## DNA-FLUOROCHROME ASSAY RESULTS

Procedures 3008, 3009, 3011

Sample ID # **58232** **M-250** Date Rec'd: **08/04/2009** P.O. # **RP2874**

Indicator Cells Inoculated: Date/Initials: 8/6/09 / BMB

Fixation: Date/Initials: 8/10/09 / JA

Staining: Date/Initials: 8/10/09 / JA

TEST/CONTROL ARTICLE:

**ES03-DL-3 #9440**

LOT# **NA**

**Wicell QA**
**WiCell Research Institute**

[redacted]

[redacted]

**DNA FLUOROCHROME ASSAY RESULTS:**

__X__ **NEGATIVE:** A reaction with staining limited to the nuclear region, which indicates no mycoplasmal contamination.

_____ **POSITIVE:** A significant amount of extranuclear staining which strongly suggests mycoplasmal contamination.

_____ **INCONCLUSIVE:**

_____ A significant amount of extranuclear staining consistent with low - level mycoplasmal contamination or nuclear degeneration.

_____ A significant amount of extranuclear staining consistent with bacterial, fungal or other microbial contaminant or viral CPE. Morphology not consistent for mycoplasmal contamination.

COMMENTS: _____

Date: 8/10/09 Results Read by: JA Date of Review: 8-10-09 Reviewed by: SeA

**WiCell Cytogenetics Report:** 001236-072009
NSCB 9440

***Report Date:*** July 28, 2009

## *Case Details:*

***Cell Line:*** *ES03-DL-3 (9440)*

***Passage #:*** *69*

***Date Completed:*** *7/28/2009*

***Cell Line Gender:*** *female*

***Investigator:*** [redacted]

***Specimen:*** *hESC on MEF feeder*

***Date of Sample:*** *7/20/2009*

***Test, Reason for:*** *DL Release Testing*

***Results:*** *46,XX*

*Completed by* [redacted], *CLSp(CG), on 7/28/2009*

*Reviewed and interpreted by* [redacted], *PhD, FACMG, on 7/28/2009*

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

***Cell:*** *S01-01*

***Slide:*** *C*

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

***Cell Results:*** *46,XX*

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

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

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

***Band Level:*** *425-500*

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

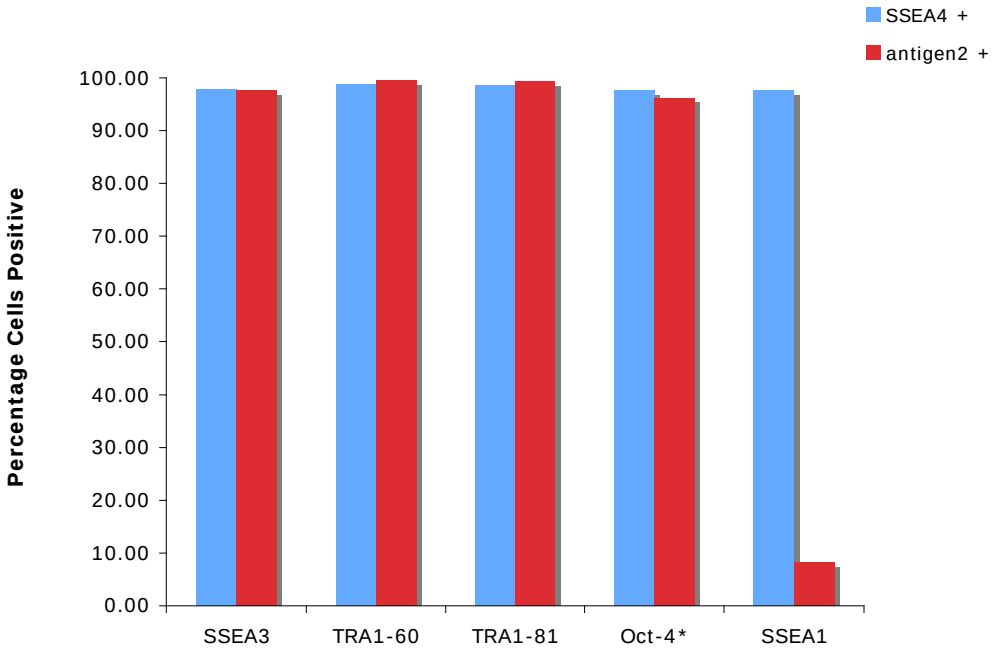
**Date:**_____
**Sent To:**_____
**Results Recorded:** _____

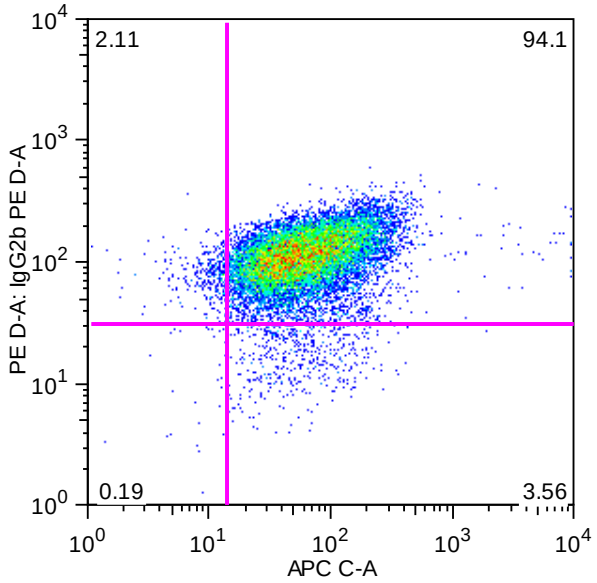
**Procedures performed:**
SOP-CH-101 SOP-CH-102
SOP-CH-103 SOP-CH-105

**Cell Line: ES03-DL-03**
**Passage 74**
**Sample ID: 9440-FAC**

**Date of**: *(mm/dd/yy)*
acquisition: 08/11/09
file creation: 08/11/09
file submission: 08/14/09

| antigen2: | SSEA4 - antigen2 + | SSEA4 + antigen2 + | SSEA4 + antigen2 - | SSEA4 - antigen2 - | ALL SSEA4 + | ALL antigen2 + |
|---|---|---|---|---|---|---|
| SSEA3 | 1.36 | 96.4 | 1.42 | 0.82 | 97.82 | 97.76 |
| TRA1-60 | 0.7 | 98.8 | 0.041 | 0.5 | 98.84 | 99.50 |
| TRA1-81 | 0.79 | 98.5 | 0.038 | 0.72 | 98.54 | 99.29 |
| Oct-4* | 2.11 | 94.1 | 3.56 | 0.19 | 97.66 | 96.21 |
| SSEA1 | 0.023 | 8.15 | 89.4 | 2.4 | 97.55 | 8.17 |




hESC
9440_Test.fcs
Event Count: 15488