Certificate of Analysis - Amended

Product Description	WA09	
Cell Line Provider	WiCell Research Institute	
Parent Material	WA09-MCB-01	
Lot Number	WA09-DL-02[1]	
Date Vialed	09-July-2007	
Passage Number	p23	
Culture Platform	Feeder Dependent	
	Medium: hES Medium	Matrix: MEFs

The following testing specifications have been met for the specified product 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	Apptec	30055	Negative	Pass
Karyotype by G-banding	WiCell	SOP-CH-003	Normal karyotype	Pass

[1]The vial is labeled in the following manner:
H9p23RD
09JUL07
SOPCC029B
-H9-2

Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans.

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.

Amendment(s):

Reason for Amendment	Date
CoA updated to include copyright information.	See Signature
CoA updated for format changes, clarification of test specifications, test method, addition of test provider, culture platform, electronic signature, and reference to WiCell instead of NSCB. Karyotype footnote removed.	10-May-2012
Added label information and included formal lot number.	02-February-2009
Original CoA	26-November-2007

Date of Lot Release	Quality Assurance Approval
20-November-2007	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: NSCB# 4685 UW HLA#: 57297 Sample Date: 10/16/07
Received Date: 10/16/07

H9-DL-2 characterization #1

Requestor: WiCell Research Institute

Test Date: 10/16/07 File Name: 071016 Report Date: 10/18/07

Sample Name: (label on tube)
NSCB# 4685 WiCell DNA054

Description: DNA Extracted by WiCell

250 ug/mL; 260/280 = 1.8

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	12,13
D7S820	6-14	9,11
D13S317	7-15	9,9
D5S818	7-15	11,12
CSF1PO	6-15	11,11
TPOX	6-13	10,11
Amelogenin	NA	X,X
TH01	5-11	9.3,9.3
vWA	11, 13-21	17,17

Comments: Based on the NSCB# 4685 DNA submitted by WI Cell dated 10/16/07 and received on 10/16/07, this sample (UW HLA# 57297) matches exactly the STR profile of the human stem cell line H9 comprising 12 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human H9 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 DNA sample submitted corresponds to the H9 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%. A preliminary copy of this report was issued via electronic mail to both [redacted] of WI Cell Research Institute on Friday, October 19, 2007.

[redacted] 10-23-07
Date
HLA/Molecular Diagnostics Laboratory

[redacted] 10/19/07
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:
[redacted]

AppTec

Report Number
752280
Page 1 of 1

WiCell Research Institute
[redacted]

August 10, 2007
P.O. #: [redacted]

STERILITY TEST REPORT

Sample Information: Human embryonic stem cell line H9 on mouse feeder layer, H9 (SOPCC029B-H9-2), 3281-APT

Date Received: July 17, 2007
Date in Test: July 26, 2007
Date Completed: August 09, 2007

Test Information: Test Code: 30744
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.02

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	0.47 mL	0.47 mL
Number Tested	1	1
Type of Media	SCD	FTM
Media Volume	200 mL	200 mL
Incubation Period	14 Days	14 Days
Incubation Temperature	20 °C to 25 °C	30 °C to 35 °C
RESULTS	1 NEGATIVE	1 NEGATIVE

QA Reviewed: [redacted] 08-10-07

Reviewed: [redacted] 08-10-07

Testing conducted in accordance with current Good Manufacturing Practices.

[redacted]

St. Paul

Report

Copy of Original

FINAL STUDY REPORT

STUDY TITLE:	**MYCOPLASMA DETECTION: "Points to Consider"**
PROTOCOL NUMBER:	30055E
TEST ARTICLE IDENTIFICATION:	SOPCC029B-H9-2, 3281-APT
SPONSOR:	WiCell Research Institute [redacted]
PERFORMING LABORATORY:	AppTec Laboratory Services [redacted]
STUDY NUMBER:	64555
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination

[redacted]

WCR01

64555

[redacted]

[redacted]

QUALITY ASSURANCE UNIT SUMMARY

STUDY: Mycoplasma Detection: "Points to Consider"

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of nonclinical laboratory studies. This study has been performed under Good Laboratory Practices regulations (FDA, 21 CFR, Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies) and in accordance to standard operating procedures and a standard protocol. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the quality and integrity of the study.

Critical Phase	Date	Study Director	Management
Staining of Coverslips	09/17/07	09/17/07	10/26/07
Final Report	10/24/07	10/25/07	10/26/07

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: [redacted] Date: 10-26-07

GOOD LABORATORY PRACTICES STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58.

The studies not performed by or under the direction of AppTec Laboratory Services, are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: [redacted] Date: 10/26/07

Professional Personnel Involved:

[redacted]

[redacted]

1.0 PURPOSE

To demonstrate that a test article consisting of a cell bank, production or seed lots, or raw materials is free of mycoplasmal contamination, according to "Points to Consider" criteria.

2.0 SPONSOR: WiCell Research Institute
[redacted]

3.0 TEST FACILITY: AppTec Laboratory Services, Inc.
[redacted]

4.0 SCHEDULING

DATE SAMPLE RECEIVED: 09/11/07
STUDY INITIATION DATE: 09/12/07
STUDY COMPLETION DATE: 10/26/07

5.0 TEST ARTICLE IDENTIFICATION: WiCell Research Institute
SOPCC029B-H9-2, 3281-APT

6.0 TEST ARTICLE CHARACTERIZATION

The Sponsor was responsible for all test article characterization data as specified in the GLP regulations. The identity, strength, stability, purity, and chemical composition of the test article were solely the responsibility of the Sponsor. The Sponsor was responsible for supplying to the testing laboratory results of these determinations and any others that may have directly impacted the testing performed by the testing laboratory, prior to initiation of testing. Furthermore, it was the responsibility of the Sponsor to ensure that the test article submitted for testing was representative of the final product that was subjected to materials characterization. Any special requirements for handling or storage were arranged in advance of receipt and the test article was received in good condition.

The test article was maintained according to the Sponsor's instructions. The Vero cells were maintained by AppTec's Cell Production Laboratory.

7.0 EXPERIMENTAL DESIGN

7.1 OVERVIEW

Whereas no single test is capable of detecting all mycoplasmal strains, freedom from mycoplasmal contamination may be demonstrated by the use of both an indirect and direct procedure.

7.2 JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Contamination of cell cultures by mycoplasma is a common occurrence and is capable of altering normal cell structure and function. Among other things, mycoplasma may affect cell antigenicity, interfere with virus replication, and mimic viral actions. Testing for the presence of mycoplasma for cell lines used to produce biologicals is recommended by the FDA, Center for Biologics Evaluation and Research (CBER) under "Points to Consider."

8.0 EXPERIMENTAL SUMMARY

The indirect method of detection allows visualization of mycoplasma, particularly non-cultivable strains, by growing the mycoplasma on an indicator cell line and then staining using a DNA-binding fluorochrome stain. The indicator cell line should be easy to grow, have a large cytoplasmic to nuclear area ratio and support the growth of a broad spectrum of mycoplasma species. The African green monkey kidney cell line, Vero, fits this description and was used in this assay. The assay was performed with negative and positive controls. Both a strongly cyto-adsorbing (*M. hyorhinis*) and a poorly cyto-adsorbing (*M. orale*) mycoplasma species were used as positive controls. Poor cyto-adsorbing mycoplasma species may not give reliable positive results when inoculated in low numbers. A second dilution of *M. orale* was used to ensure cyto-adsorption. Staining the cultures with DNA binding fluorochrome allows for the detection of mycoplasma based on the staining pattern observed. Only the cell nuclei demonstrate fluorescence in the negative cultures but nuclear and extra-nuclear fluorescence is observed in positive cultures.

Direct cultivation is a sensitive and specific method for the detection of mycoplasma. The agar and broth media employed supply nutrients necessary for the growth of cultivable mycoplasmas. These media also supply a source of carbon and energy, and favorable growth conditions. The direct assay was performed with both negative and positive controls. A fermentative mycoplasma (*M. pneumoniae)* and a non-fermentative mycoplasma *(M. orale)* were used as positive controls. The procedure employed in this study is based on the protocol described in the 1993 Attachment # 2 to the "Points To Consider" document, as recommended by the FDA, Center for Biologics Evaluation and Research (CBER).

9.0 TEST MATERIAL AND PREPARATION

9.1 TEST ARTICLE IDENTIFICATION:

Test Article Name:	SOPCC029B-H9-2, 3281-APT
Lot/Batch #:	Not Given
Stability (Expiration):	Not Given
Storage Conditions:	Ultracold (≤ -60°C)
Safety Precautions:	BSL-1
Intended Use/Application:	Distribution lot cells from MCB cells

9.2 TEST SAMPLE PREPARATION

The test article was thawed in a water bath at 37 ± 2°C and 1:5 and 1:10 dilutions were prepared in sterile phosphate buffered saline (PBS). 1.0 mL of the undiluted sample, the 1:5 and 1:10 dilutions were then inoculated onto each of two (2) coverslips (per sample/dilution) containing Vero cells. The coverslips were incubated in incubator E770 for 1-2 hours at 37 ± 1°C / 5 ± 2% CO_2 and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E770 at 37 ± 1°C / 5 ± 2% CO_2. After three days of incubation, the coverslips were fixed, stained, and then read using an epifluorescent microscope.

0.2 mL of the undiluted test article was then inoculated onto each of two SP-4 agar plates, and 10.0 mL was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth. The plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for a minimum of 14 days.

The broth flask was incubated aerobically at 36 ± 1°C, and subcultured onto each of two SP-4 agar plates (0.2 mL/plate) on Days 3, 7, and 14. These subculture plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for a minimum of 14 days. The broth flask was read each working day for 14 days. The SP-4 agar plates (Day 0) were read after 14 days of incubation. The SP-4 broth subculture plates (Days 3, 7, and 14) were read after 14 days incubation.

9.3 CONTROLS AND REFERENCE MATERIALS

9.3.1 Sterile SP-4 broth served as the negative control for both the direct and indirect assays.

9.3.2 Positive Controls

a. Indirect Assay

a.1 Strongly cyto-adsorbing species - *M. hyorhinis* GDL (ATCC #23839) at 100 or fewer colony forming units (CFU) per inoculum.

a.2 Poorly cyto-adsorbing species - *M. orale* (ATCC #23714) at 100 or fewer CFU and at approximately 100 ID_{50} per inoculum.

b. Direct Assay

b.1 Nonfermentative mycoplasma species - *M. orale* (ATCC #23714) at 100 or fewer CFU per inoculum.

b.2 Fermentative mycoplasma species - *M. pneumoniae* FH (ATCC #15531) at 100 or fewer CFU per inoculum.

9.3.3 Control Preparation

a. Negative Controls

a.1 1.0 mL of sterile SP-4 broth was inoculated onto each of two (2) coverslips containing Vero cells to serve as the negative control in the indirect assay.

a.2 0.2 mL of SP-4 broth was inoculated onto each of two (2) SP-4 agar plates to serve as the negative control in the direct assay. 10.0 mL of SP-4 broth was inoculated into a 75 cm^2 flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

WiCell Cytogenetics Report: NSCB# 8534

Report Date: May 09, 2012

Case Details:

Cell Line: *NSCB#8534 (H9)*

Passage #: *NSCB*

Date Completed: *11/19/2007*

Cell Line Gender: *female*

Investigator: *National Stem Cell Bank*

Specimen: *hESC on MEF feeder*

Date of Sample: *11/9/2007*

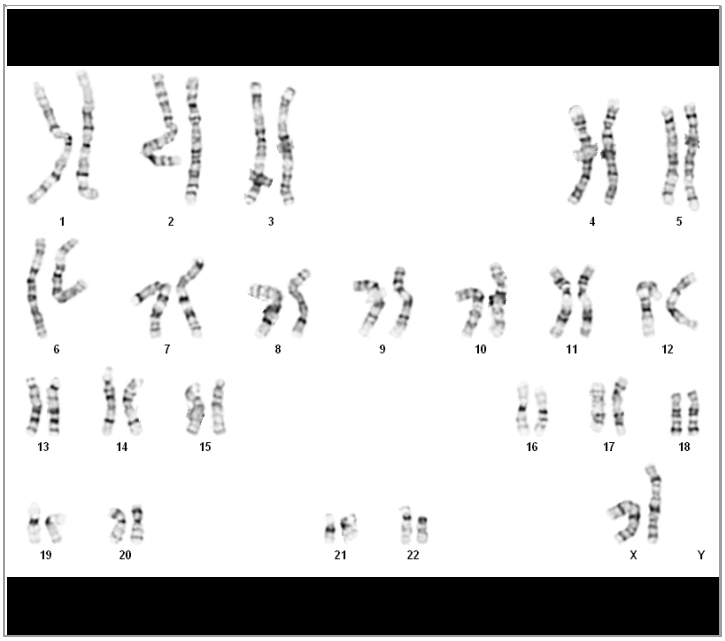
Tests,Reason for: *NSCB Characterization*

Results: *46,XX*

Completed by [redacted], *CLSp(CG), on 11/19/2007*

Reviewed and interpreted by [redacted] *PhD, FACMG, on 11/19/2007*

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



Cell: *S01-04*

Slide: *B*

Slide Type: *Karyotyping*

Cell Results: *Karyotype: 46,XX*

# of Cells Counted: *20*

# of Cells Karyotyped: *4*

# of Cells Analyzed: *8*

Band Level: *450-550*

Results Transmitted by Fax / Email / Post **Date:**_____

Sent By:_____ **Sent To:**_____