Certificate of Analysis - Amended

Fast Track Distribution Lot

Product Description	BG03 NSCB FT Distribution lot
Cell Line Provider	Novocell (San Diego, CA, USA)
Distribution Lot Number	BG03-FTDL-1
Date Vialed	21-March-2008
Passage Number	24
Culture Method	SOP-CC-020B, SOP-CC-030B, SOP-CC-037A
Cryopreservation Method	SOP-CC-035D

The following testing specifications have been met for the specified product lot:

Test Description	Test Method	Test Specification	Result
Post-Thaw Viable Cell Recovery	SOP-CH-305A	Viable cells recovered	Pass
Identity by STR	SOP-CH-302B	Positive identity	Pass
Sterility	SOP-CH-304A	No contamination detected	Pass
Mycoplasma	SOP-CH-020A	No contamination detected	Pass
Karyotype by G-banding	SOP-CH-003B	Normal karyotype	Pass

Electronic versions of this certificate of analysis (CoA) complete with electronic copies of individual reports, results, and procedures are available on our website, www.wicell.org. There are also archived CoAs for past cell lots.

Cells distributed by the National Stem Cell Bank are intended for research purposes only and are not intended for use in humans. These cells have undergone testing and are not known to harbor pathogens. However, 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. The NSCB is not responsible for damages or injuries that may result from the use of these cells.

Please visit the technical service portion of the website for assistance with your human ES Cells. The knowledgeable technical support staff can assist with embryonic stem cell culture concerns, training, and any other customer service concerns you may encounter.

Amendment(s):

Reason for Amendment	Date
CoA updated to include copyright information and electronic signature. Links updated.	See signature
Original CoA	13-June-2008

Date of Lot Release	Quality Assurance Approval
13-June-2008	12/30/2013 X AMC AMC Quality Assurance Signed by: [redacted]

UW Health
University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory
D4/231; (608) 263-8815
600 Highland Avenue
Madison, WI 53792-2472

Short Tandem Repeat Analysis*

Sample Report: 4584-STR BG03-FTDL-1

UW HLA#: not accessioned

Sample Date: 05/13/08
Lab Received 05/14/08

Requestor: WiCell Research Institute

Test Date: 05/19/08

File Name: 080520

Report Date: 05/26/08

Sample Name: (label on tube) 4584-STR

Description: WI Cell Cytogenetics provided genomic DNA
237 ug/mL

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

Comments: Based on the 4584-STR DNA submitted by WI Cell dated 05/13/08 and received on 05/14/08, this sample matches exactly the STR profile of the human stem cell line BG03 comprising 11 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human BG03 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. This result suggest that the 4584-STR DNA sample submitted corresponds to the BG03 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%. A preliminary copy of this report was issued via electronic mail to WI Cell Research Institute on Monday, May 26, 2008.

HLA/Molecular Diagnostics Laboratory

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

WiCell Research Institute

April 17, 2008
P.O. #: RP1781

STERILITY TEST REPORT

Sample Information: 3: BG03-FTDL-1, hES cells (BG03FTDL1 P24 MW)

Date Received: April 01, 2008
Date in Test: April 02, 2008
Date Completed: April 16, 2008

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

TEST PARAMETERS	PRODUCT	
Approximate Volume Tested	0.5 mL	0.5 mL
Number Tested	2	2
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	2 NEGATIVE	2 NEGATIVE

QA Reviewed: Page 1 Signed _____

Reviewed: Page 1 Signed _____

Testing conducted in accordance with current Good Manufacturing Practices.

St. Paul

Report

WuXi AppTec

FINAL STUDY REPORT

STUDY TITLE:	**MYCOPLASMA DETECTION: "Points to Consider"**
PROTOCOL NUMBER:	30055E
TEST ARTICLE IDENTIFICATION:	BG03-FTDL-1
SPONSOR:	WiCell Research Institute
PERFORMING LABORATORY:	WuXi AppTec, Inc. 2540 Executive Drive St. Paul, MN 55120
STUDY NUMBER:	106019
RESULT SUMMARY:	Considered **negative** for mycoplasma contamination

Reference PO # RP1849

WCR01

106019

erika@wicell.org

WuXi AppTec

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
Inoculation of Coverslips	05/09/08	05/09/08	06/13/08
Final Report	06/11/08	06/11/08	06/13/08

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

Quality Assurance Auditor: Tamara Fossum Date: 6/13/08
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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 WuXi AppTec, Inc., are exempt from this Good Laboratory Practice Statement and include characterization and stability of the test compound(s)/test article.

Study Director: Sheri J. Zielinski Date: 6/13/08
ki

Professional Personnel Involved:

	Vice President of St. Paul Operations
	Manager, Mycoplasma Testing Laboratory
, BS	Study Director
\A	Client Relations Manager

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

3.0 TEST FACILITY: WuXi AppTec, Inc.
2540 Executive Drive
St. Paul, MN 55120

4.0 SCHEDULING

DATE SAMPLE RECEIVED: 05/07/08
STUDY INITIATION DATE: 05/08/08
STUDY COMPLETION DATE: 06/13/08

5.0 TEST ARTICLE IDENTIFICATION: WiCell Research Institute; BG03-FTDL-1

6.0 SAMPLE STORAGE

Upon receipt by the Sample Receiving Department, the test samples were placed in a designated, controlled access storage area ensuring proper temperature conditions. Test and control article storage areas are designed to preclude the possibility of mix-ups, contamination, deterioration or damage. The samples remained in the storage area until retrieved by the technician for sample preparation and/or testing. Unused test samples remained in the storage area until the study was completed. Once completed, the remaining samples were discarded or returned as requested by the Sponsor.

7.0 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 Vero cells were maintained by WuXi AppTec's Cell Production Laboratory.

8.0 EXPERIMENTAL DESIGN

8.1 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.

8.2 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."

9.0 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).

10.0 TEST MATERIAL PREPARATION

10.1 Test Article Identification:

Test Article Name:	BG03-FTDL-1
General Description:	hES Cells
Number of Aliquots used:	1 x 15 mL
Stability (Expiration):	Not Given
Storage Conditions:	Ultracold (< -60°C)
Safety Precautions:	BSL-1

10.2 Test Sample Preparation

The test article was thawed in a water bath at 37 ± 2°C and 1:5 and 1:10 dilutions of the test article 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.

10.3 Controls and Reference Materials

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

10.3.2 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.

10.3.3 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.

b. Positive Controls

b.1 *M. hyorhinis, M. orale,* and *M. pneumoniae* were diluted to less than 100 CFU per inoculum in sterile SP-4 broth. 1.0 mL of *M. hyorhinis* and *M. orale* at less than 100 CFU/mL was inoculated onto each of two (2) coverslips containing Vero cells. 1.0 mL of *M. orale* at 100 ID_{50} CFU per inoculum was also inoculated onto each of two (2) coverslips containing Vero cells. These coverslips served as the positive controls in the indirect assay.

b.2 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 cell cultures were fixed, stained, and then read using an epifluorescent microscope.

b.3 0.2 mL of *M. orale* and *M. pneumoniae* at less than 100 CFU/plate was inoculated onto each of two (2) SP-4 agar plates. 10.0 mL of *M. orale* and *M. pneumoniae* at less than 10 CFU/mL (≤100 CFU/inoculum) were each inoculated into a 75 cm^2 flask containing 50 mL of sterile SP-4 broth.

b.4 The agar plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C for 14 days. The broth cultures were incubated aerobically at 36 ± 1°C for a minimum of 14 days and were read each working day for 14 days. On Days 3, 7, and 14, 0.2 mL from each broth culture flask was subcultured onto each of two (2) SP-4 agar plates. These subculture plates were placed in an anaerobic GasPak system and incubated at 36 ± 1°C. The subculture plates were observed microscopically for the presence of mycoplasma colonies after a minimum of 14 days incubation.

c. See Section 15.0, Results, for the results of these controls.

11.0 DATA ANALYSIS

The results of this study were based on visual observations, therefore, no data analysis was required.

12.0 STATISTICAL METHODS

The results of this study were qualitative, therefore, no statistical analysis was required.

13.0 EVALUATION CRITERIA

Final evaluation of the validity of the assay and test article results was based upon the criteria listed below and scientific judgment.

13.1 Indirect Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY INDIRECT ASSAY

CONTROLS	MYCOPLASMA FLUORESCENCE OBSERVED (AT LEAST ONE COVERSLIP REQUIRED FOR THE EVALUATION)
Negative Control	-
M. hyorhinis	+
M. orale (≤100 CFU)	+/-*
M. orale (100 ID_{50})	+

*Mycoplasma must be observed for at least one dilution of the poorly cyto-adsorbing mycoplasma species *M. orale*.

13.2 Direct Assay

DETECTION OF MYCOPLASMA CONTAMINATION BY DIRECT ASSAY

	NEGATIVE CONTROL	*M. PNEUMONIAE*	*M. ORALE*
Broth (Color change or turbidity change)	-	+ / -	+ / -
Agar Day 0 (at least one plate)	-	+	+
Agar Day 3, 7, 14 (at least one plate on one day)	-	+	+
Results	-	+	+

14.0 TEST EVALUATION

14.1 Indirect Assay

Hoechst stain will bind to most DNA containing organisms and organelles present in the culture; this includes indicator cell nuclei, prokaryotes including mycoplasma and cell debris. The source of DNA is determined by the staining pattern. The indicator cell nuclei fluoresce brightly and are generally 10-20 μm in diameter. Mycoplasma fluorescence is less intense, is extra-nuclear and typically appears as small round bodies approximately 0.3 μm in diameter.

14.2 Direct Assay

Change in color or turbidity of broth culture can be an indicator of the presence of mycoplasma growth. Fermentative mycoplasma produce acid from the carbohydrates in the medium causing the pH of the medium to drop and the broth to turn yellow in color. Nonfermentative mycoplasma produce ammonia by arginine hydrolysis causing the pH to rise and the broth to turn red. In general, growth of mycoplasma can cause the broth to become turbid. These changes must be confirmed by agar plate subculture or DNA-staining since changes in the appearance of the broth culture can also be caused by the properties of the inoculum.

Mycoplasma colonies grow down into the agar causing the center of the colony to appear opaque and the peripheral surface growth to appear translucent. These "fried-egg" colonies vary in size, 10-500 μm, and can be readily observed unstained using a light microscope.

14.3 Indirect Assay and Direct Assay Results Interpretation

IF:	TEST ARTICLE				
Mycoplasmal fluorescence	-	+	+/-	+/-	-
Broth (Color change or turbidity change)	-	+/-	+/-	+/-	+*
Agar - Day 0 (at least one plate)	-	+/-	+/-	+	-
Agar - Day 3, 7, 14 (at least one plate on one day)	-	+/-	+	+/-	-
THEN: OVERALL FINAL RESULT	**-**	**+**	**+**	**+**	**-**

*A change in the appearance of a broth culture must be confirmed by positive subculture plate(s).

14.4 Positive Results

The test article is considered positive if the direct assay (agar or broth media procedure) or the indirect assay (indicator cell culture procedure) show evidence of mycoplasma contamination and resemble the positive controls for the procedure.

14.5 Negative Results

The test article is considered as negative if both the direct assay (agar and broth media procedure) and the indirect assay (indicator cell culture procedure) show no evidence of mycoplasma contamination and resemble the negative control for each procedure.

15.0 RESULTS

Indirect Assay and Direct Assay Results

	INDIRECT	DIRECT		OVERALL
		BROTH FLASKS	AGAR PLATES	
Test Article: BG03-FTDL-1	Negative	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative
M. hyorhinis	Positive			Positive
M. orale	Positive	Positive	Positive	Positive
M. pneumoniae		Positive	Positive	Positive

For the indirect assay, the coverslips for the undiluted test article were read and determined negative.

16.0 ANALYSIS AND CONCLUSION

16.1 The results of the negative and positive controls indicated the validity of this test.

16.2 These findings indicated that the test article, BG03-FTDL-1, is considered negative for the presence of mycoplasma contamination.

WiCell Cytogenetics Report: 000532-050208
NSCB 4584

Report Date: May 09, 2008

Case Details:

Cell Line: *BG03-FTDL-1*

Passage #: *27*

Date Completed: *5/7/2008*

Cell Line Gender: *female*

Investigator: *National Stem Cell Bank*

Specimen: *hESC on MEF feeder*

Date of Sample: *5/2/2008*

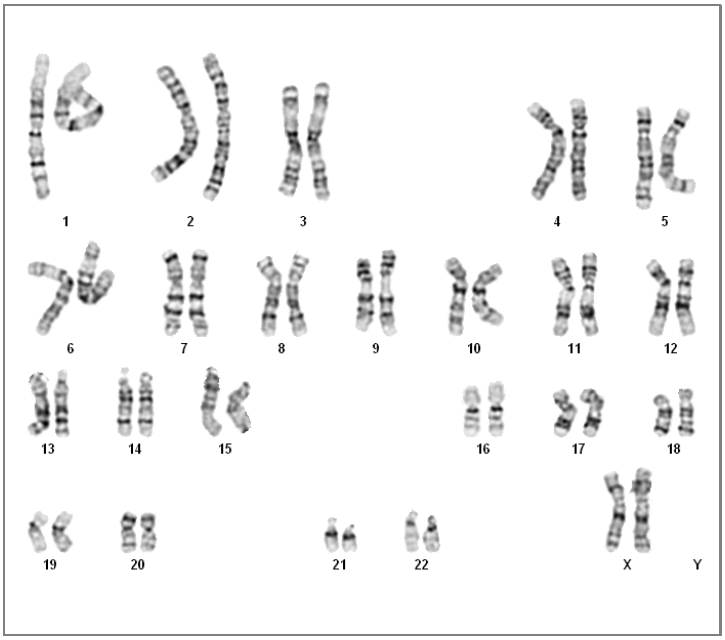
Tests,Reason for: *Fast-track distribution lot testing*

Results: *46,XX*

Completed by CS, CLSp(CG), on 5/7/2008

Reviewed and interpreted by KDM, PhD, FACMG, on 5/7/2008

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



Cell: *S01-01*

Slide: *A*

Slide Type: *Karyotyping*

Cell Results: *Karyotype: 46,XX*

# of Cells Counted: *20*

# of Cells Karyotyped: *4*

# of Cells Analyzed: *8*

Band Level: *450-525*

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

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