



Thaw and Culture Details

Cell Line Name	ES02
WiCell Lot Number	ES02-DL-03
Parent Material	ES02-MCB-01
Provider	ES Cell International
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	p66 These cells were cultured for 65 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 Vialied	29-August-2008
Vial Label	ES02-DL-3 p66 LK 29 AUG 2008 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. 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, ≤ 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	Apptec	30055	No contamination detected	Pass
Flow Cytometry for ESC Marker Expression	UW Flow Cytometry Laboratory	SOP-CH-101 SOP-CH-102 SOP-CH-103 SOP-CH-105	Report - no specification	See report



Date of Lot Release	Quality Assurance Approval
20-February-2009	<p style="text-align: right;">7/14/2020</p> <p>X AA</p> <p>AA Quality Assurance Signed by: Arntz, Andy</p>

Short Tandem Repeat Analysis*

**Sample Report: 6696-STR
(ES02-DL-3)**

UW HLA#: 59723

Sample Date: 10/21/08
Received Date: 10/21/08

Requestor: WiCell Research Institute

Test Date: 10/23/08

File Name: 081024

Report Date: 10/28/08

Sample Name: (label on tube) **6696-STR****Description:** DNA Extracted by WiCell
255 ug/mL; 260/280 = 1.8

Locus	Repeat #	STR Genotype
D16S539	5, 8-15	9,12
D7S820	6-14	9,11
D13S317	7-15	8,11
D5S818	7-15	11,11
CSF1PO	6-15	11,11
TPOX	6-13	8,12
Amelogenin	NA	X,X
TH01	5-11	7,9
vWA	11, 13-21	18,20

Comments: Based on the 6696-STR DNA dated and received on 10/21/08 from WI Cell, this sample (UW HLA# 59723) matches exactly the STR profile of the human stem cell line **ES02** comprising 14 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human ES02 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 6696-STR DNA sample submitted corresponds to the ES02 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%. These results were communicated via phone to the Cytogenetics laboratory of the WiCell Research Institute on Monday, October 27, 2008. A preliminary copy of this report was issued via electronic mail to the WI Cell Research Institute on Friday, October 31, 2008.

* 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
788629
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WiCell Research Institute

October 07, 2008
P.O. #:

STERILITY TEST REPORT

Sample Information: hES Cells / iPS Cells
1: WA13C-DL-2
2: BG03-DL-2
3: ES02-DL-3
4: iPS(IMR90)-4-DL-1
5: WA07-DL-2

Date Received: September 17, 2008
Date in Test: September 22, 2008
Date Completed: October 06, 2008

Test Information: Test Codes: 30744, 30744A
Immersion, USP / 21 CFR 610.12
Procedure #: BS210WCR.201

QA Reviewed: 

Reviewed:

Testing conducted in accordance with current Good Manufacturing Practices.

Report Number
788629
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WiCell Research Institute

October 07, 2008
P.O. #:

STERILITY TEST REPORT

Sample Information: hES Cells / iPS Cells
3: ES02-DL-3

Date Received: September 17, 2008
Date in Test: September 22, 2008
Date Completed: October 06, 2008

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	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



FINAL STUDY REPORT

STUDY TITLE: MYCOPLASMA DETECTION:
"Points to Consider"

PROTOCOL NUMBER: 30055G

TEST ARTICLE IDENTIFICATION: ES02 - DL - 3

SPONSOR: WiCell Research Institute

PERFORMING LABORATORY: WuXi AppTec. Inc.

STUDY NUMBER: 113374

RESULT SUMMARY: Considered **negative** for mycoplasma contamination

Reference PO #



Study Number: 113374
Protocol Number: 30055G

WiCell Research Institute
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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.

<u>Critical Phase</u>	<u>Date</u>	<u>Study Director</u>	<u>Management</u>
Dilution of Controls	11/14/08	11/14/08	12/23/08
Final Report	12/22/08	12/22/08	12/23/08

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

Quality Assurance Auditor: _____

Date: 12/23/08

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: _____

Date: 12/23/08

Professional Personnel Involved:

1.0 PURPOSE

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

2.0 SPONSOR: WiCell Research Institute

3.0 TEST FACILITY: WuXi AppTec, Inc.

4.0 SCHEDULING

DATE SAMPLE RECEIVED: 11/06/08
STUDY INITIATION DATE: 11/12/08
STUDY COMPLETION DATE: 12/23/08

5.0 TEST ARTICLE IDENTIFICATION: WiCell Research Institute
ES02 - DL - 3

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 mycoplasma strains, freedom from mycoplasma 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:	ES02 - DL - 3
Lot/Batch #:	Not Given
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
Intended Use/Application:	Not Given

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 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 / $5 \pm 2\%$ CO₂ 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 / $5 \pm 2\%$ CO₂. 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 cm² flask containing 50 mL of SP-4 broth. The plates were incubated anaerobically 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 incubated anaerobically 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 ID₅₀ 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 cm² flask containing 50 mL of SP-4 broth to serve as the negative control in the direct assay.

b. Positive Controls

b.1 *M. hyorhinae*, *M. orale*, and *M. pneumoniae* were diluted to less than 100 CFU per inoculum in sterile SP-4 broth. 1.0 mL of *M. hyorhinae* 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 ID₅₀ 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 E950 for 1-2 hours at 37 ± 1 °C / 5 ± 2% CO₂ and then 2.0 mL of EMEM, 8% Fetal Bovine Serum (FBS) was added to each coverslip. The coverslips were returned to incubator E950 at 37 ± 1 °C / 5 ± 2% CO₂. 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 cm² flask containing 50 mL of sterile SP-4 broth.

b.4 The agar plates were incubated anaerobically 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 incubated anaerobically 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 COVERSIP REQUIRED FOR THE EVALUATION)
Negative Control	-
<i>M. hyorhinis</i>	+
<i>M. orale</i> (≤ 100 CFU)	+/-*
<i>M. orale</i> (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	<i>M. PNEUMONIAE</i>	<i>M. ORALE</i>
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
		DAY 0 AGAR PLATES	BROTH FLASKS AND ASSOCIATED SUBCULTURE PLATES	
Test Article: ES02 - DL - 3	Negative	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative
<i>M. hyorhinis</i>	Positive			Positive
<i>M. orale</i>	Positive	Positive	Positive	Positive
<i>M. pneumoniae</i>		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 indicate the validity of this test.

16.2 These findings indicate that the test article, ES02 - DL - 3, is considered negative for the presence of mycoplasma contamination.

17.0 DEVIATIONS: None.

18.0 AMENDMENTS: None.

19.0 RECORD RETENTION

An exact copy of the original final report and all raw data pertinent to this study will be stored at WuXi AppTec, Inc., 2540 Executive Drive, St. Paul, MN 55120. It is the responsibility of the Sponsor to retain a sample of the test article.

20.0 TECHNICAL REFERENCES

- 20.1** Barile, Michael F. and McGarrity, Gerard J. (1983). "Isolation of Mycoplasmas from Cell Culture by Agar and Broth Techniques." *Methods in Mycoplasmaology*, Vol II, ed. J.G. Tully and S. Razin. (New York: Academic Press) pp. 159-165.
- 20.2** Del Giudice, Richard A. and Joseph G. Tully. 1996. "Isolation of Mycoplasma from Cell Cultures by Axenic Cultivation Techniques," ed. J.G. Tully and S. Razin, *Molecular and Diagnostic Procedures in Mycoplasmaology*, Vol. II (New York: Academic Press).
- 20.3** McGarrity, Gerard J. and Barile, Michael F. 1983. "Use of Indicator Cell Lines for Recovery and Identification of Cell Culture Mycoplasmas," ed. J.G. Tully and S. Razin, *Methods in Mycoplasmaology*, Vol. II (New York: Academic Press).
- 20.4** Masover, Gerald and Frances Becker. 1996. "Detection of Mycoplasma by DNA Staining and Fluorescent Antibody Methodology," ed. J.G. Tully and S. Razin, *Molecular and Diagnostic Procedures in Mycoplasmaology*, Vol. II (New York: Academic Press).
- 20.5** Schmidt, Nathalie J. and Emmons, Richard W. 1989. "Cell Culture Procedures for Diagnostic Virology," ed. Nathalie J. Schmidt and Richard W. Emmons, 6th ed., *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections* (Washington: American Public Health Association).
- 20.6** U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER). 1993. "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals."

Report Date: October 14, 2008

Case Details:

Cell Line: ES02-DL-3-J (6696)

Passage #: NSCB

Date Completed: 10/14/2008

Cell Line Gender: Female

Investigator: National Stem Cell Bank

Specimen: hESC on MEF feeder

Date of Sample: 10/10/2008

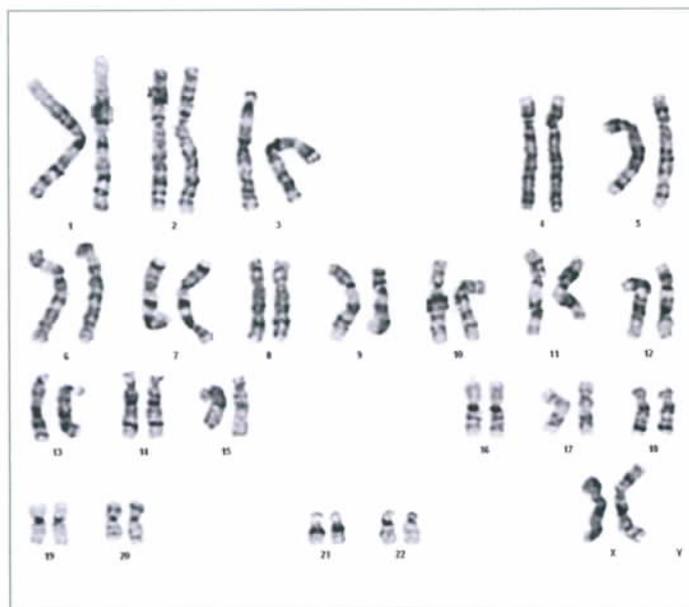
Tests, Reason for: NSCB - Distribution Lot Release

Results: 46,XX

Completed by: _____, CLSp(CG), on 10/14/2008

Reviewed and interpreted by: _____, PhD, FACMG, on 10/14/2008

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: 3

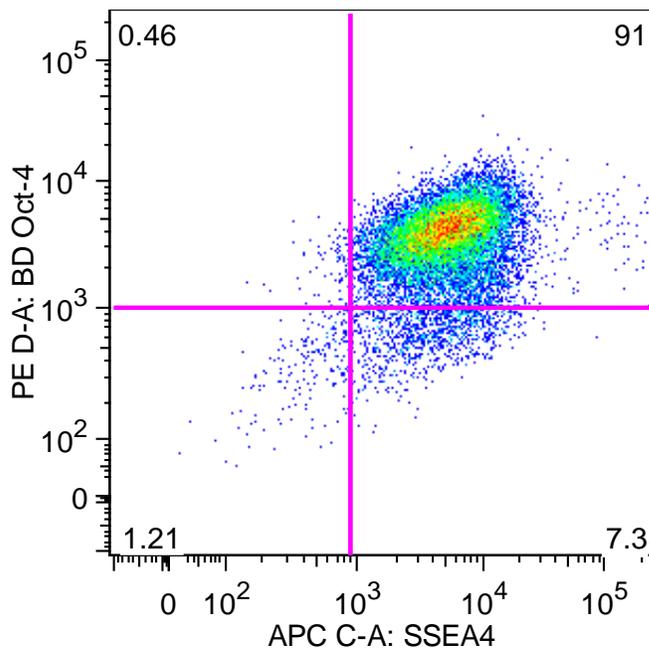
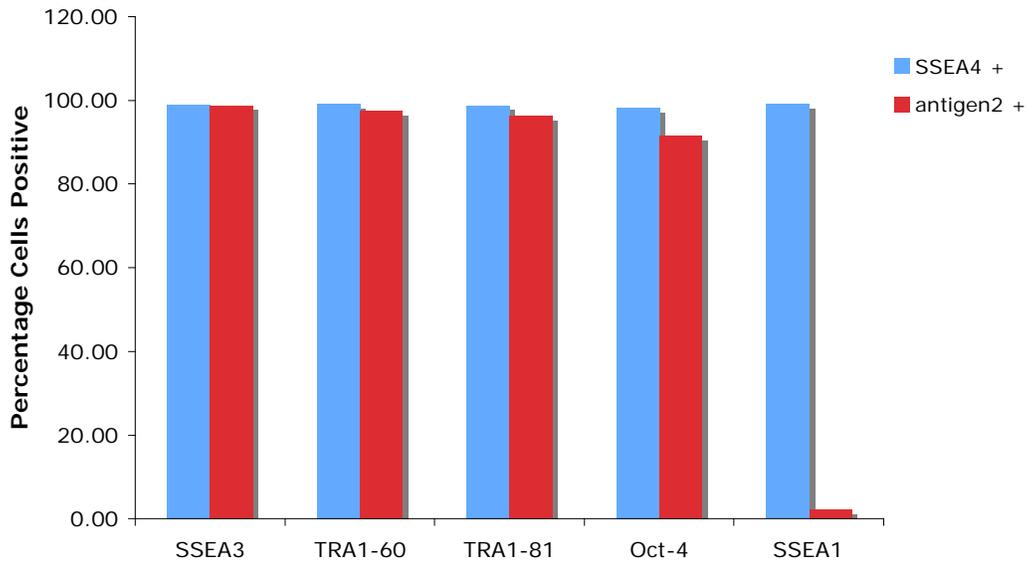
of Cells Analyzed: 8

Band Level: 450-525

Results Transmitted by Fax / Email / Post
Sent By: _____

Date: _____
Sent To: _____

<u>antigen2:</u>	SSEA4 - <u>antigen2 +</u>	SSEA4 + <u>antigen2 +</u>	SSEA4 + <u>antigen2 -</u>	SSEA4 - <u>antigen2 -</u>	ALL <u>SSEA4 +</u>	ALL <u>antigen2 +</u>
SSEA3	0.12	97.80	1.21	0.82	99.01	98.92
TRA1-60	0.09	97.50	1.73	0.72	99.23	97.59
TRA1-81	0.10	96.20	2.72	0.96	98.92	96.30
Oct-4*	0.46	91.00	7.30	1.21	98.30	91.46
SSEA1	0.09	2.06	97.1	0.75	99.16	2.15



*PE-conjugated Oct-3/4 from BD Biosciences was used (cat #560186).