Product Information and Testing

Product Information

Product Name	NSC-H9
Lot Number	WB0305
Parent Material	NSC-H9-DB0002
Depositor	Buck Institute for Resarch on Aging
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 4 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: NSC Medium
	Matrix: Geltrex
Protocol	WiCell recommends using the depositor protocol.
Passage Number	p19 These cells were cultured for 18 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	09-June-2014
Vial Label	NSC-H9 WB0305 p19 09JUN14
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
Post-Thaw Viable Cell Recovery	WiCell	SOP-CH-305	≥ 15 Undifferentiated Colonies, ≤ 30% Differentiation and recoverable attachment after passage	Pass
Identity by STR	UW Molecular Diagnostics Laboratory	PowerPlex 16 HS System by Promega	Consistent with known profile	Pass
Sterility	Biotest Laboratories	ST/07	Negative	Pass
Mycoplasma	WiCell	SOP-QU-004	Negative	Pass
Karyotype by G-banding	WiCell	SOP-CH-003	Expected karyotype	Pass

Date of Lot Release	Quality Assurance Approval
15-May-2015	5/15/2015 X AMK AMK Quality Assurance Signed by: [redacted]

UW Health

University of Wisconsin
Hospital and Clinics

Short Tandem Repeat Analysis*

Molecular Diagnostics Laboratory
600 Highland Avenue
Clinical Sciences Center (D4/211)
Madison, WI 53792-2472

Samples Report:
(1) 10991-STR 176.1 ng/uL (260/280=1.86)

DNA Extracted by WiCell Research Institute

Requestor:
WiCell Research Institute (Quality Assurance)
Phone: 608-577-6625
qa@wicell.org
Contact: Jessica Martin

Sample Date(s): 08/07/14
Receive Date(s): 08/07/14
Assay Date(s): 08/13/14
File Name(s): 140813 CLN
Report Date(s): 08/19/14

STR Locus	STR Genotype Repeat #	(1)
FGA	16–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2, 45.2, 46.2	26,28
TPOX	6-13	10,11
D8S1179	7-18	8,14
vWA	10-22	17,17
Amelogenin	X,Y	X,X
Penta_D	2.2, 3.2, 5, 7-17	9,13
CSF1PO	6-15	11,11
D16S539	5, 8-15	12,13
D7S820	6-14	9,11
D13S317	7-15	9,9
D5S818	7-16	11,12
Penta_E	5-24	11,14
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	13,13
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38	30,30
TH01	4-9,9.3,10-11,13.3	9.3,9.3
D3S1358	12-20	13,16

Comments: Based on the 10991-STR DNA dated and received on 08/07/14 from WI Cell, this sample (Label on Tube: 10991-STR) exactly matches and further defines the STR profile of the human stem cell line WA09 (H9) comprising 24 allelic polymorphisms across the 15 STR loci analyzed. No STR polymorphisms other than those corresponding to the human NSC-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 10991-STR DNA sample submitted corresponds to the WA09 (H9) 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] 10/14/14
Molecular Diagnostics Laboratory — Date

[redacted] 10/14/14
Molecular Diagnostics Laboratory — Date

* Testing 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

Sterility Report

Biotest Laboratories, Inc.
Making life-saving products possible

CORRECTED REPORT

WiCell Research Institute, Inc.
WiCell Quality Assurance
[redacted]

BIOTEST SAMPLE #	14071020
VALIDATION #	NG
TEST PURPOSE	NG

PRODUCT	10996, 10997, 10999, 11000, WA07-WB0292 11004, WA09-WB0299 11005, NSC-H9-WB0305 11007, WISe-OGFP-H2CH-DB0035 11008
PRODUCT LOT	NA
STERILE LOT	NA
STERILIZATION LOT	NA
STERILIZATION DATE	NA
STERILIZATION METHOD	NA
SAMPLING BLDG / ROOM	NA

BI LOT	NA
BI EXPIRATION DATE	NA
DATE RECEIVED	2014-07-18
TEST INITIATED	2014-07-18
TEST COMPLETED	2014-08-01

REFERENCE: Processed according to LAB-003: Sterility Test Procedure

Eight (8) products were each divided between 40 mL TSB and 40 mL FTG. The samples were then cultured at 20-25 C and 30-35 C respectively and were monitored for a minimum of 14 days.

- ☒ USP
- ☐ BI Manufacturers Specifications
- ☐ Other

RESULTS	# POSITIVES	# TESTED	POSITIVE CONTROL	NEGATIVE CONTROL
Sterile	0	8	NA	2 Negatives

COMMENTS: Report revised due to typo in Product Name.

REVIEWED BY [redacted] DATE 04AUG14

Mycoplasma Report

Testing Performed by WiCell

CDM/Quarantine/LRT 6-20-2014

FORM SOP-QU-004.01

Version B

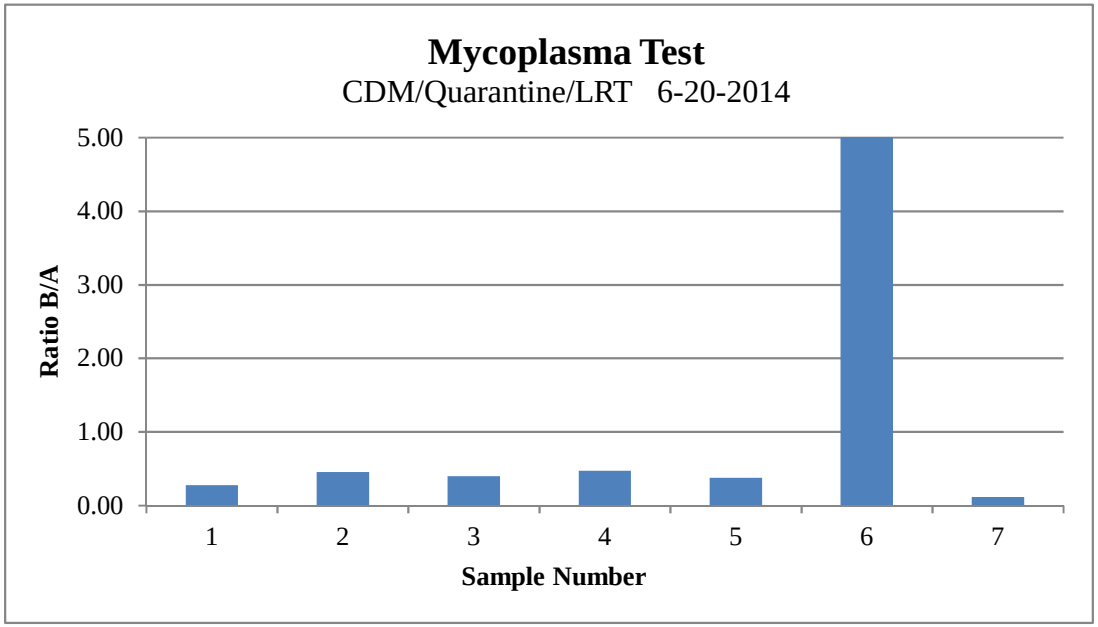
Edition 01

Assay performed and reported by: MWS

Reviewed by: DF

Equipment ID: Berthold 539

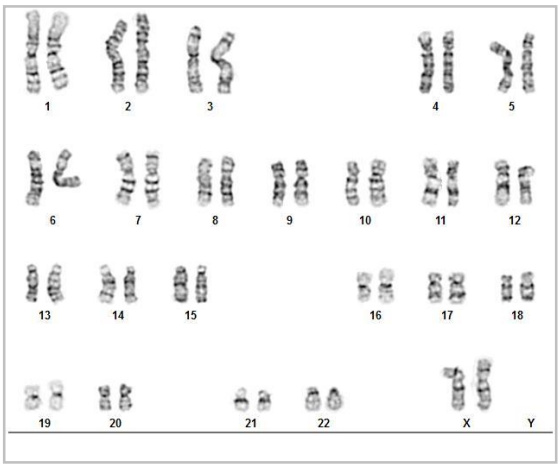
	Sample Number and ID	Reading A		A	Reading B		B	Ratio	Mycoplasma Results	Comments/Suggestions
		A1	A2	Average	B1	B2	Average	B/A		
1	NSC-H9-WB0305 10983	259	248	253.5	73	66	69.5	0.27	Negative	
2	IISH9i-GM12365-DB0032 10990	239	237	238	114	101	107.5	0.45	Negative	
3	IISH10i-GM020920-DB0033 10993	388	387	387.5	153	153	153	0.39	Negative	
4	IISH7i-GM01715-DB0030 10992	175	167	171	85	76	80.5	0.47	Negative	
5	IISH8-GM07125-DB0031 10984	197	199	198	78	71	74.5	0.38	Negative	
6	Positive (+) Control	208	210	209	13874	13940	13907	66.54	Positive	
7	Negative (-) Control	437	442	439.5	53	48	50.5	0.11	Negative	



Chromosome Analysis Report: 014522

Date Reported: Friday, July 18, 2014
Cell Line: NSC-H9-WB0305 10991
Passage#: 20
Date of Sample: 7/7/2014
Specimen: NSC
Results: 46,XX[18]
Nonclonal findings: 47,XX,+2 47,XX,+mar

Cell Line Gender: Female
Reason for Testing: lot release testing
Investigator: [redacted], WiCell CDM



Cell: 24
Slide: 4
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyotyped: 6
Band Resolution: 350 - 400

Interpretation:

This is a normal karyotype. No clonal abnormalities were detected at the stated band level of resolution.

There are two unrelated nonclonal findings, listed above. Nonclonal findings likely result from technical artifact, but may be due to a developing clonal abnormality or to low-level mosaicism.

Completed by: [redacted] MS, CG(ASCP)
Reviewed and Interpreted by: [redacted], PhD, FACMG

A signed copy of this report is available upon request.

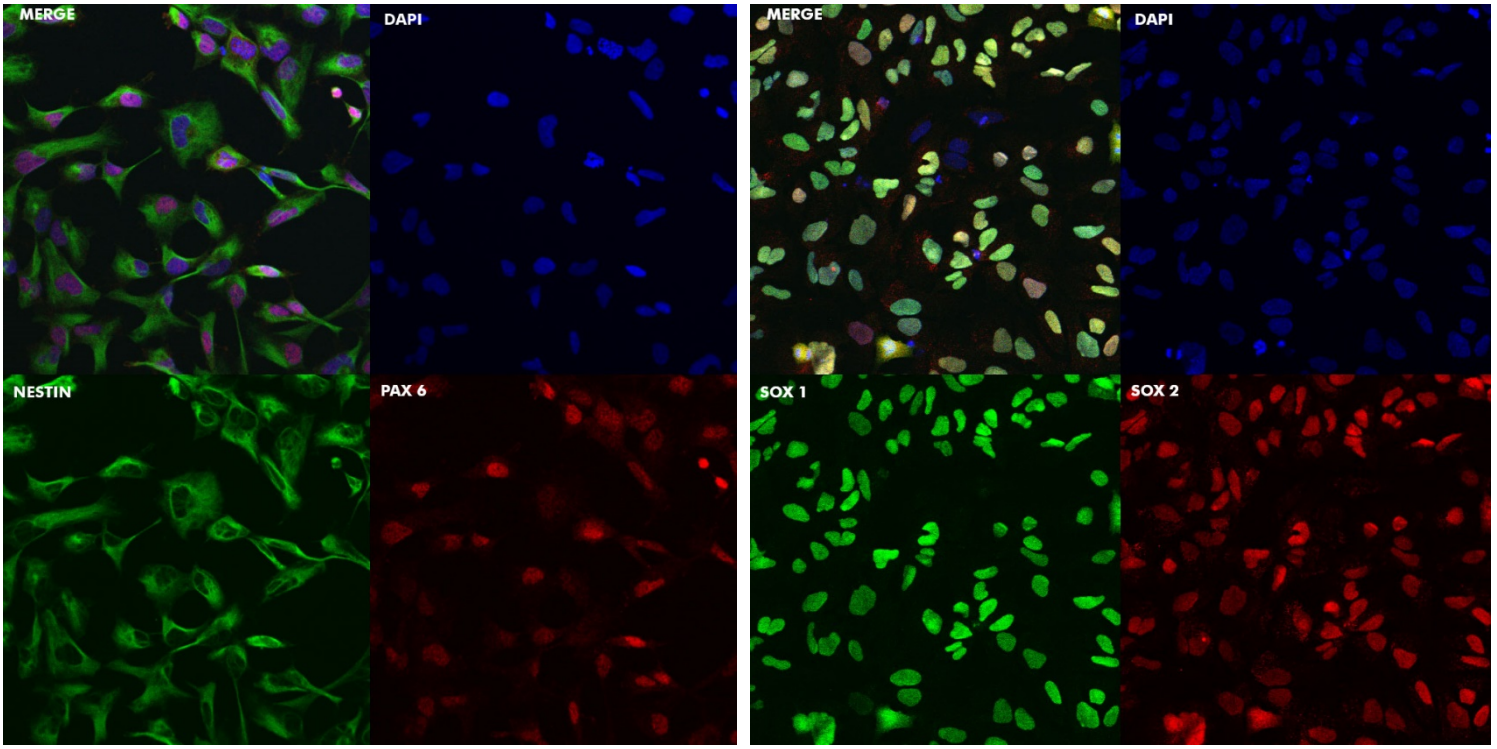
Date:_____ **Sent By:**____ **Sent To:**_____ **QC Review By:** ____

Limitations: This assay allows for microscopic visualization of numerical and structural chromosome abnormalities. The size of structural abnormality that can be detected is >3-10Mb, dependent upon the G-band resolution obtained from this specimen. For the purposes of this report, band level is defined as the number of G-bands per haploid genome. It is documented here as "band level", i.e., the range of bands determined from the four karyograms in this assay. Detection of heterogeneity of clonal cell populations in this specimen (i.e.,mosaicism) is limited by the number of metaphase cells examined, documented here as "# of cells counted". This assay was conducted solely for listed investigator/institution. The results may not be relied upon by any other party without the prior written consent of the Director of the WiCell Cytogenetics Laboratory. The resu lts of this assay are for research use only. If the results of this assay are to be used for any other purpose, contact the Director of the WiCell Cytogenetics Laboratory. Unless otherwise mutually agreed in writing, the services provided to you hereunder by WiCell Research Institute, Inc. ("WiCell") are governed solely by WiCell's Terms and Conditions of Service, found at www.wicell.org/privacyandterms. Any terms you may attach to a purchase order or other document that are inconsistent, add to, or conflict with WiCell's Terms and Conditions of Service are null and void and of no legal force or effect.

Confirmation staining of WB 0305 NSC-H9 Neural Stem Cells

METHODS: Cells were thawed into two matrigel coated wells of a six-well plate using the WiCell NSC Maitanace, Freezing and Thawing protocol. Once the cells became confluent the NSC-H9 Cells (p21) were passaged onto matrigel coated cover slips in a 24 well plate. Once the cells reached ~70% confluency they were fixed and stained using Life Technologies Cat # A24354 Human Neural Stem Cell Immunocytochemistry Kit (antibodies in table below). Images were collected using the Nikon A1R-Si confocal laser scanning microscope with a 20x dry objective.

Primary antibodies	Secondary antibodies
anti-NESTIN (host: mouse) A24345	Alexa Fluor® 488 donkey anti-mouse
anti-PAX6 (host: rabbit) A24340 Alexa Fluor®	Alexa Fluor® 555 donkey anti-rabbit
anti-SOX1 (host: goat) A24347 Alexa Fluor®	Alexa Fluor® 488 donkey anti-goat
anti-SOX2 (host: rabbit) A24339	Alexa Fluor® 555 donkey anti-rabbit



[redacted], Waisman iPSC core, November 18, 2016

NSC-H9-WB0305 Verification of Neural Stem Cell. 09Dec16 JKG