Product Information and Testing - Amended

Product Information

Product Name	NSC-H14
Lot Number	WB0195
Depositor	Buck Institute for Research on Aging
Banked by	WiCell
Thaw Recommendation	Thaw 1 vial into 3 wells of a 6 well plate.
Culture Platform	Feeder Independent
	Medium: NSC Medium
	Matrix: Geltrex
Protocol	WiCell recommends using the depositor protocol included in the CoA and testing results packet.
Passage Number	p21 These cells were cultured for 20 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	05-October-2012
Vial Label	WB0195 H14NSC P21 JB 05OCT12
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	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

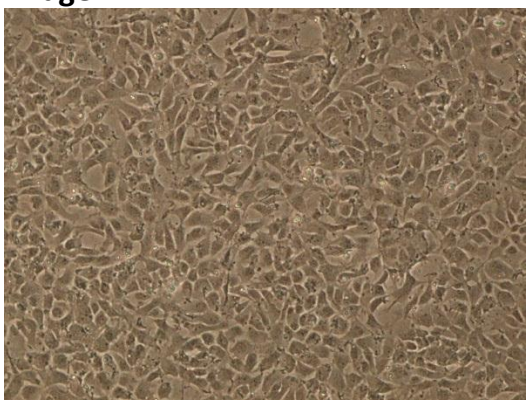
Amendment(s):

Reason for Amendment	Date
CoA sterility testing provider and method updated.	See signature
CoA updated product name.	11-February-2013
Original CoA	28-January-2013

Date of Lot Release	Quality Assurance Approval
28-January-2013	4/22/2013 X AMC AMC Quality Assurance Signed by: [redacted]

Protocol:	NSC maintenance, freezing and thawing

Image:



Caption:
This is an image of NSC culture on geltrex surface.

Flow Chart:

Coating dishes with Geltrex
Enzymatic Passage of NSC
Maintenance of NSC
Cryopreservation of NSC
Thawing of NSC

Instructions:

Coating dishes with Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix

1. Thaw Geltrex 5 ml in original bottle overnight at 4^0C.
2. Aliquot to 1 ml/tube, store at -20°C (tips, pipets and tubes should be prechilled)
3. Thaw the tube 1-2 hours on ice or O/N at 4 degree
4. Dilute this 1:50 in cold Neurobasal media and coat dishes (2 ml/60mm, 1 ml/35 mm).
5. Incubate RT for 1 hr.

Enzymatic Passage of NSCs

1. Carefully aspirate medium from 60 mm dish containing confluent NSCs.
2. Add 1-2 mL of pre-warmed accutase onto the dish, place it in the cell culture incubator and for 3-5 min until cells detach.
3. Collect detached NSCs using P-1000 pipette and place cells in 15 mL conical tube. Rinse the dish once again with 3 mL of pre-warmed neurobasal medium and collect in the same tube.
4. Centrifuge cells at 580 x g for 4 min
5. Aspirate supernatant carefully and re-suspend cells in 3 mL of NSC medium.

Note: Cells from one confluent 60 mm dish shall be split onto three new 60 mm dishes (split 1:3). Approximately 2-2.5 million of cells per 60 mm dish will give a desirable density.

6. Aspirate geltrex solution from freshly coated dishes, add 4 mL of NSC medium into each plate and transfer 1 mL of NSCs (from step 5) into each plate.

7. Distribute NSCs evenly.
8. Incubate at 37°C/ 5% CO_2 and change medium every other day.

Maintenance of NSC

1. Observe and assess NSC culture every day. Generally media should be change every other day.
2. Condition aliquot of fresh NSC media in the water bath at 37^0C.
3. Carefully aspirate media from the cell culture dish.
4. Slowly add fresh media on to the dish allowing fluid to slither along the wall, avoid pouring media directly onto the cells.

Cryopreservation of NSCs

This protocol can be applied for NSCs collected from several dishes.

1. Carefully aspirate medium from 60 mm dish containing confluent NSCs.
2. Add 1-2 mL of pre-warmed accutase onto the dish, place it in the cell culture incubator and for 3-5 min until cells detach.
3. Wash off NSCs from the dish using P-1000 pipette and place cells in 15 mL conical tube. Rinse the dish once again with 3 mL of pre-warmed neurobasal medium and collect in the same tube.
4. Centrifuge cells at 580 x g for 4 min
5. Re-suspend cells in a small volume of NSC-FREEZE-A medium (1 mL or more if multiple dishes are combined) and count them.
6. Dilute cells using the same medium to obtain desired cell density (4-5 millions cells/mL).
7. Carefully add an equal volume of NSC-FREEZE-B medium containing 20% DMSO under constant swirling (final cell density 2-2.5 millions cells/mL). Mix gently 2-3 times by pipetting.
8. Alternatively to steps 5-7: Resuspend cells in 500 µl of CryoStem Freezing medium to obtained cell density of 2-2.5 millions cells/mL.
9. Aliquot into cryogenic vials (1 mL/vial).
10. Immediately freeze at -80˚C using isopropanol contraption and transfer vials into liquid nitrogen tank the following day.

Thawing NSC from Frozen Stocks.

1. Thaw cells in 37°C water bath
2. Transfer cells to 15 mL conical tube containing 5 mL of pre-warmed Neurobasal medium under constant swirling. Wash the cryo-vial with additional 1 mL of medium and transfer to the same 15 mL tube.
3. Centrifuge cells at 580 x g for 4 min
4. Aspirate supernatant and re-suspend cells in 5 mL of NSC medium
5. Plate onto geltrex coated dishes (2 millions/35 mm dish)
6. Distribute evenly and incubate at 37°C/ 5% CO_2
7. Change medium every other day

Materials:

Neurobasal medium SFM, Life Technologies 21103049
B27 supplement, Life Technologies 17504044
MEM NEAA, Life Technologies 11140050
GlutaMax-I, Life Technologies 35050061
Rec Hu bFGF, Stemgent 03-002
DMSO, Sigma
Accutase, Life Technologies
Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix,
Life Technologies A1413202
D60, D35 CT Dishes Corning
Cryovial Corning
Conical tubes 15 ml, 50 ml Corning
CryoStem Freezing Medium, Stemgent 01-013-51

Media :

1. NSC Expansion Medium (NSC medium): Neurobasal medium supplemented with B27 (1x), 2 mM MEM NEAA MEM, 2 m*M* GlutaMAX-I, Rec Hu bFGF 10 ng/mL,
   For 100 mL: Neurobasal 96 mL, B27 supplemet 2 mL, MEM NEAA 1 mL, GlutaMAX-I 1 mL, Rec Hu bFGF 10 µL.
2. NSC freezing medium A (NSC-FREEZE-A): Neurobasal medium supplemented with B27 (1x), 2 m*M* MEM NEAA, 2 m*M* GlutaMAX-I. For 100 mL: Neurobasal 96 mL, B27 supplement 2 mL,MEM NEAA 1 mL, GlutaMAX-I 1 mL,
3. NSC freezing medium B (NSC-FREEZE-B): Neurobasal medium supplemented with 20% DMSO, 2 m*M* MEM NEAA, 2 m*M* GlutaMAX-I. For 100 mL: Neurobasal 78 mL, DMSO 20 mL, MEM NEAA 1 mL, GlutaMAX-I 1 mL

FAQs:

Xianmin Zeng Laboratory at the Buck Institute for Research on Aging, Novato, CA

Last Updated: 02/03/2012

Contributed By: Anna Maria Swistowska, amswistowska@buckinstitute.org

UW Health

University of Wisconsin
Hospital and Clinics

Histocompatibility/Molecular Diagnostics Laboratory

[redacted]

Short Tandem Repeat Analysis*

Sample Report: 10668-STR | Label on Tube: 10668-STR | Sample Date: 01/04/13
Received Date: 01/04/13

Requestor: WiCell Research Institute

Test Date: 01/09/13 | File Name: 130109 | Report Date: 01/11/13

Sample Name: (label on tube)
10668-STR

Description: DNA Extracted by WiCell

228.52 ug/mL; 260/280 = 1.94

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

Comments: Based on the 10668-STR DNA dated and received on 01/04/13 from WiCell, this sample (UW HLA# Label on Tube: 10668-STR 01/04/13) exactly matches the STR profile of the human stem cell line WA14 (H14) comprising 14 allelic polymorphisms across the 8 STR loci analyzed. No STR polymorphisms other than those corresponding to the human WA14 (H14) 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 10668-STR DNA sample submitted corresponds to the WA14 (H14) 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]
Molecular Diagnostics Laboratory

[redacted]
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

STERILITY REPORT

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

BIOTEST SAMPLE # 13030760

VALIDATION # NG

TEST PURPOSE NG

PRODUCT NAME	Please see packing slip under product name.		
PRODUCT LOT	NA		
STERILE LOT	NA	BI LOT	NA
STERILIZATION LOT	NA	BI EXPIRATION DATE	NA
STERILIZATION DATE	NA	DATE RECEIVED	2013-03-15
STERILIZATION METHOD	NA	TEST INITIATED	2013-03-15
SAMPLING BLDG / ROOM	NA	TEST COMPLETED	2013-03-29

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

11 products were 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 ☐ Non-Sterile ☐ NA	0	11	NA	2 Negatives

COMMENTS NA

REVIEWED BY [redacted] DATE 29MAR13

WiCell Research Institute

Packing Slip

[redacted]

Sent to:
Sterility Testing Services
BiotestLabs, Sterility Testing Services
[redacted]

Date:
12Mar13

Product Name	Condition
[redacted] NSC-WA0195 #10726 [redacted]	-80

13030760 SLK
MAR 15 2013

Mycoplasma Report

Testing Performed by WiCell

RLRT-10668/JB 12-6-2012

FORM SOP-QU-004.01

Version B

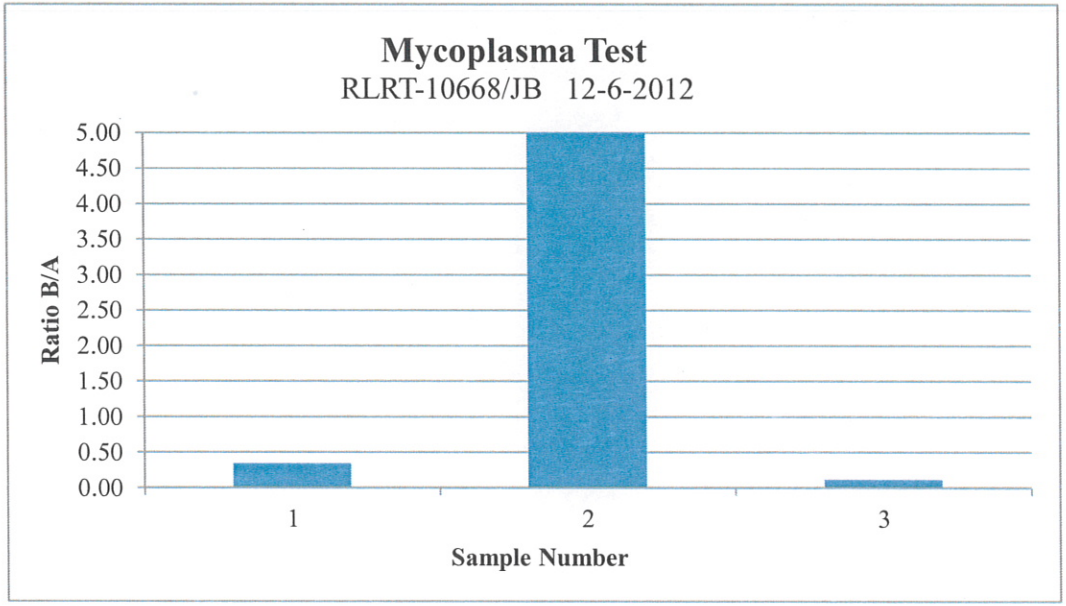
Edition 01

Assay performed and reported by: MW

Reviewed by: JB

Equipment ID: 539 Berthold

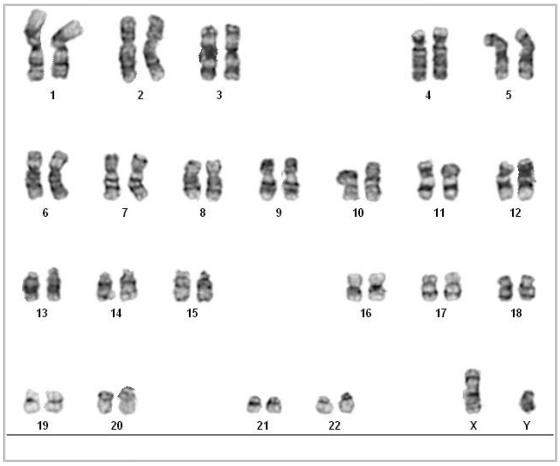
	Sample Number and ID	Reading A		A	Reading B		B	Ratio	Mycoplasma Results	Comments/Suggestions
		A1	A2	Average	B1	B2	Average	B/A		
1	H14NSC-WB0195 -10668	196	199	197.5	69	69	69	0.35	Negative	
2	Positive (+) Control	204	202	203	13834	13878	13856	68.26	Positive	
3	Negative (-) Control	302	298	300	40	33	36.5	0.12	Negative	



Chromosome Analysis Report: 009491

Date Reported: Friday, December 21, 2012
Cell Line: H14NSC-WB0195 10668
Passage#: 22
Date of Sample: 12/7/2012
Specimen: Neural Stem Cell
Results: 46,XY

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



Cell: 9
Slide: 1
Slide Type: Karyotype

Total Counted: 20
Total Analyzed: 8
Total Karyotyped: 4
Band Resolution: 350 - 400

Interpretation:

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

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

A signed copy of this report is available upon request.

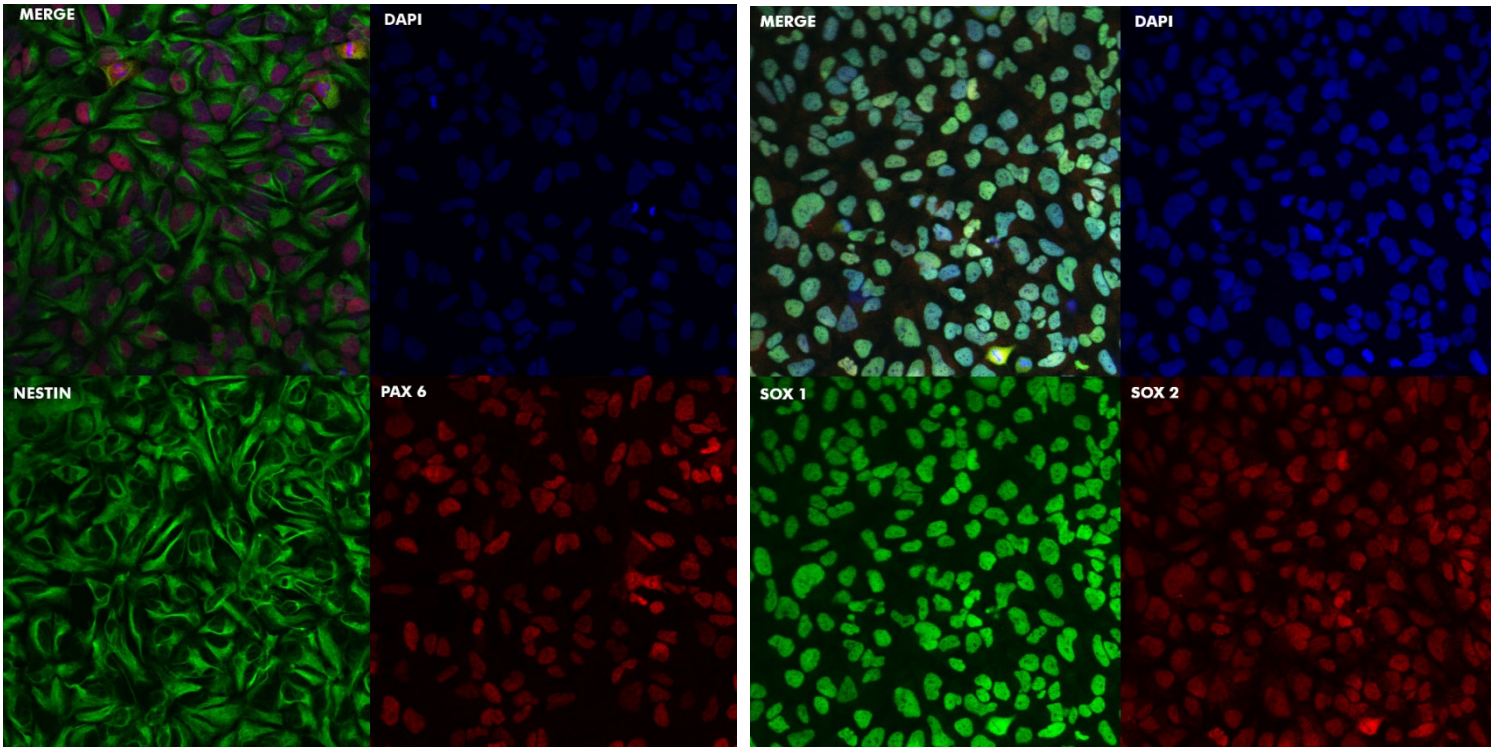
Date:_____ **Sent By:**____ **Sent To:**_____ **QC Review By:** ____

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".

Confirmation staining of WB 0195 H14 NCS Neural Stem Cells

METHODS: Cells were thawed into two matrigel coated wells of a six-well plate using the WiCell NSC Maintenance, Freezing and Thawing protocol. Once the cells became confluent the H14 NSC Cells (p23) were passaged onto matrigel coated cover slips in a 24 well plate. Once the cells reached ~80% 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-H14-WB0195 Verification of Neural Stem Cell. 09Dec16 JKG