

Certificate of Analysis

NIA Aging Cell Repository

Human induced Pluripotent Stem Cell (iPSC) Line: AG27711*B

Diagnosis	Alzheimer Disease		
Parental cell line mutation			
Parental cell type, cell line ID	Fibroblast, AG08259		
Sex	Male		
Reprogramming method	Sendai viral vectors containing OCT4, SOX2, KLF4, and CMYC		
Passage number at freeze	P15		
Culture media	DMEM/F12 + 20% KOSR + 10ng/ml bFGF		
eder or Matrix substrate CF1 MEFs on 0.1% Gelatin			
Recommended passage method and split ratio	TrypLE Express; 1:8 every 5 days		
iPSC line establishment publication(s)			

The following testing specifications have been met for this product lot:

Test Description	Test Method	Test Specification	Result
Post-Thaw Cell Viability	Colony doubling	Colony formation and diameter doubling within 5 days	Pass
Sterility	Growth on agar and broth Negative		Pass
Mycoplasma	qRT-PCR Negative		Pass
Alkaline Phosphatase Staining	Cell staining >80% cells with positive staining		Pass
Identity Match	STR (THO-1, D22S417, D10S526, vWA31, D5S592, and FES/FPS)		
Genomic Integration of Episomal Plasmid	enomic PCR using plasmid specific primers nd endogenous FBXO1 control No plasmid specific sequence amplified using 100 ng gDNA template		N/A
Detection of Sendai Virus Genome and Transgene	qRT-PCR using SEV specific primers	rimers No detection of SEV genome or transgenes	
Surface Antigen Expression of Stem Cell Markers	nmunostaining and flow cytometric detection >80% expression of SSEA4		Pass
Differentiation Potential	Embryoid body (EB) formation and gene expression		
Cytogenomics	G-banding, Affymetrix Human SNP Array 6.0	0 46,XY[23].arr(1-22)x2,(X,Y)x1	

*Note:

Digisha Patel	02/18/2020	Christine Grandizio	02/27/2020
Technician, Stem Cell Laboratory	Date	Manager, Stem Cell Laboratory	Date

Disclaimer: iPSC lines distributed by Coriell Institute for Medical Research may differ from one passage or expansion to another.

Form 1701-15 Rev E-110519: NIA Certificate of Analysis AG27711*B

403 Haddon Avenue, Camden, NJ 08103-1505 | (856) 966-7377 TEL | (856) 964-0254 FAX | catalog.coriell.org



Post-Thaw Cell Viability

One distribution lot vial of the cell line was thawed and placed in culture. Cultures were observed daily. Colonies were photographed upon first appearance, then 4 days later. Colonies must double in diameter within 5 days. The area for 5 colonies was measured using CellSens software on the Olympus IX50 microscope at 40x magnification. The average area is reported here.

Day	Average area (µm ²)		
1	10,901		
5	184,784		

Colony area increased by 17 fold.

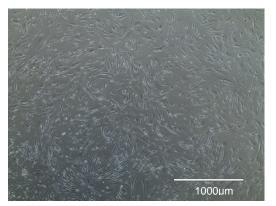


Figure 1A. Colonies post thaw (Day 1)

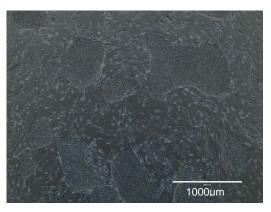


Figure 1B. Colonies 4 days after first observation (Day 5)

Alkaline Phosphatase Staining

Cells were stained using the StemTAG[™] Alkaline Phosphatase Staining Kit from CellBiolabs, Inc.

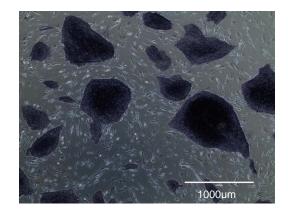


Figure 2. iPSC colonies showing alkaline phosphatase activity

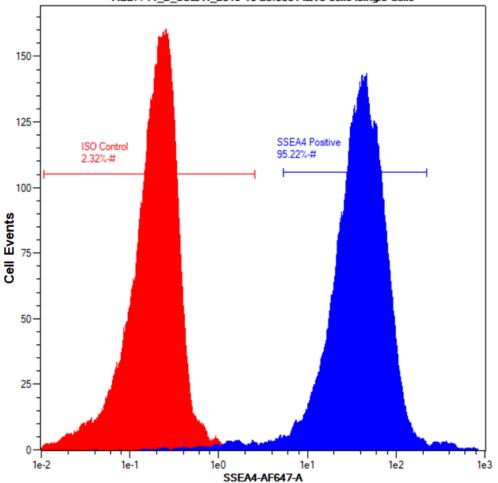
Form 1701-15 Rev E-110519: NIA Certificate of Analysis AG27711*B

403 Haddon Avenue, Camden, NJ 08103-1505 | (856) 966-7377 TEL | (856) 964-0254 FAX | catalog.coriell.org



Surface Antigen Expression of Stem Cell Markers

Undifferentiated cells are stained for stage specific embryonic antigen 4 (SSEA4) which is expressed on the surface of undifferentiated human pluripotent stem cells. Cells were analyzed using the MACSQuant Flow Cytometer by Miltyeni Biotec. More than 80% of cells should stain with antibodies specific for SSEA4.



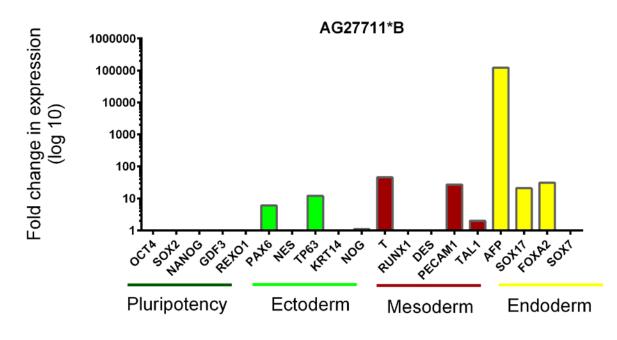
AG27711_B_SSEA4_2019-10-23.0001\Live cells\Single Cells

Figure 3. Representative histogram of SSEA4 positive population showing an overlay of isotype stained control (red) and SSEA4 positive population (blue)



Differentiation Potential

Cells are differentiated by embryoid body (EB) formation to assess pluripotency. RNA is extracted and gene expression is measured by quantitative RT-PCR. Ct values are adjusted to the endogenous housekeeping gene GAPDH. Relative gene expression is shown as the fold difference in expression compared to undifferentiated cells. Expression of at least one gene per germ layer should increase by 2 fold or higher.



Gene	Fold change	Gene	Fold change	Gene	Fold change	Gene	Fold change
OCT4	0	PAX6	6	Т	46	AFP	123252
SOX2	0	NES	0	RUNX1	0	SOX17	21
NANOG	0	TP63	12	DES	0	FOXA2	31
GDF3	0	KRT14	0	PECAM1	27	SOX7	0
REXO1	0	NOG	1	TAL1	2		

Figure 4. Fold change in expression of pluripotency genes and tri-lineage specific genes

Note: Negative values are set as 0. Calculations are performed using the $2^{-\Delta\Delta CT}$ method. (*Livak KJ, Schmittgen TD. Methods. 2001 Dec;25(4):402-8.PMID:11846609*)



Cytogenomics

Microarray	Affymetrix Human SNP Array 6.0
Cytogenetic Banding Technique	G-banding
Passage at Analysis	P17
Metaphase Cells Counted	25
Metaphase Cells Analyzed	25
Metaphase Cells Karyotyped	7
Short ISCN	46,XY[23].arr(1-22)x2,(X,Y)x1

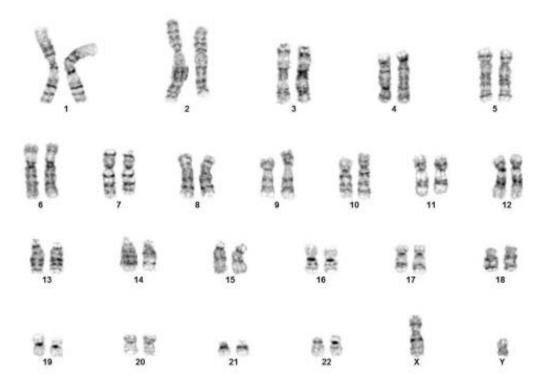


Figure 5. G-banding karyogram

Form 1701-15 Rev E-110519: NIA Certificate of Analysis AG27711*B

403 Haddon Avenue, Camden, NJ 08103-1505 | (856) 966-7377 TEL | (856) 964-0254 FAX | catalog.coriell.org