

Certificate of Analysis

**NIGMS Human Genetic Cell Repository**

Human induced Pluripotent Stem Cell (iPSC) Line: **GM28249\*B**

<b>Diagnosis</b>	Canavan Disease
<b>Parental cell line mutation</b>	ASPA; Heterozygous for c.914 C>A (p.Ala305GLu), c.527 C>A (p.Gly176Asp), and c.693 C>T (p.Tyr231Ter); HEXA; Homozygous for p.Arg249Trp
<b>Parental cell type, cell line ID</b>	Fibroblast, GM00059
<b>Sex</b>	Female
<b>Reprogramming method</b>	Sendai viral vectors containing OCT4, SOX2, KLF4, and CMYC
<b>Passage number at freeze</b>	P15
<b>Culture media</b>	mTeSR1™
<b>Feeder or Matrix substrate</b>	Matrigel®
<b>Recommended passage method and split ratio</b>	Versene; 1:8 every 5-7 days*
<b>iPSC line establishment publication(s)</b>	

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

Test Description	Test Method	Test Specification	Result
<b>Post-Thaw Cell Viability</b>	Colony doubling	Colony formation and diameter doubling within 5 days	Pass
<b>Sterility</b>	Growth on agar and broth	Negative	Pass
<b>Mycoplasma</b>	qRT-PCR	Negative	Pass
<b>Alkaline Phosphatase Staining</b>	Cell staining	>80% cells with positive staining	Pass
<b>Identity Match</b>	STR (THO-1, D22S417, D10S526, vWA31, D5S592, and FES/FPS)	Match parental cell line	Pass
<b>Genomic Integration of Episomal Plasmid</b>	Genomic PCR using plasmid specific primers and endogenous FBXO1 control	No plasmid specific sequence amplified using 100 ng gDNA template	N/A
<b>Detection of Sendai Virus Genome and Transgene</b>	qRT-PCR using SEV specific primers	No detection of SEV genome or transgenes	Pass
<b>Surface Antigen Expression of Stem Cell Markers</b>	Immunostaining and flow cytometric detection	>80% expression of SSEA4	Pass
<b>Differentiation Potential</b>	Embryoid body (EB) formation and gene expression	Minimum of 1 gene per germ layer expressed 2 fold or higher	Pass
<b>Cytogenomics</b>	G-banding	46,XX[20]	Pass

\*Note: Recover cells in 2 wells of 6-well plate.

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Technician, Stem Cell Laboratory      Date

Christine Grandizio  
.....  
Manager, Stem Cell Laboratory      Date

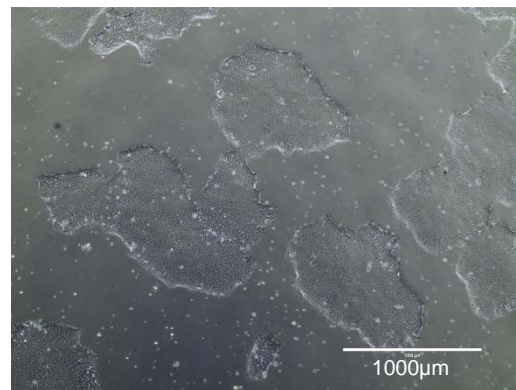
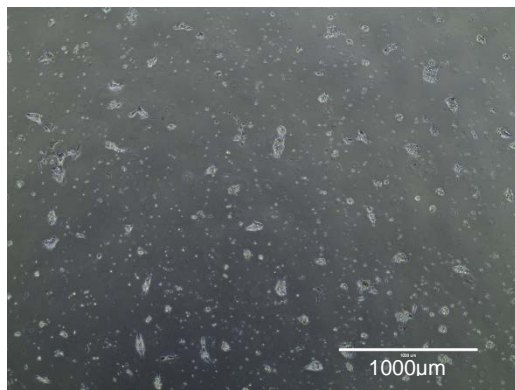
Disclaimer: iPSC lines distributed by Coriell Institute for Medical Research may differ from one passage or expansion to another.  
Form 1701-07 Rev Q-050720: NIGMS HGCR Certificate of Analysis GM28249\*B

## 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 ( $\mu\text{m}^2$ )
1	10,575
5	899,398

Colony area increased by 85 fold.

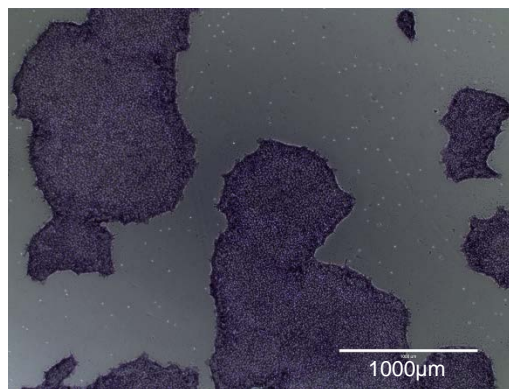


**Figure 1A.** Colonies post thaw (Day 1)

**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.

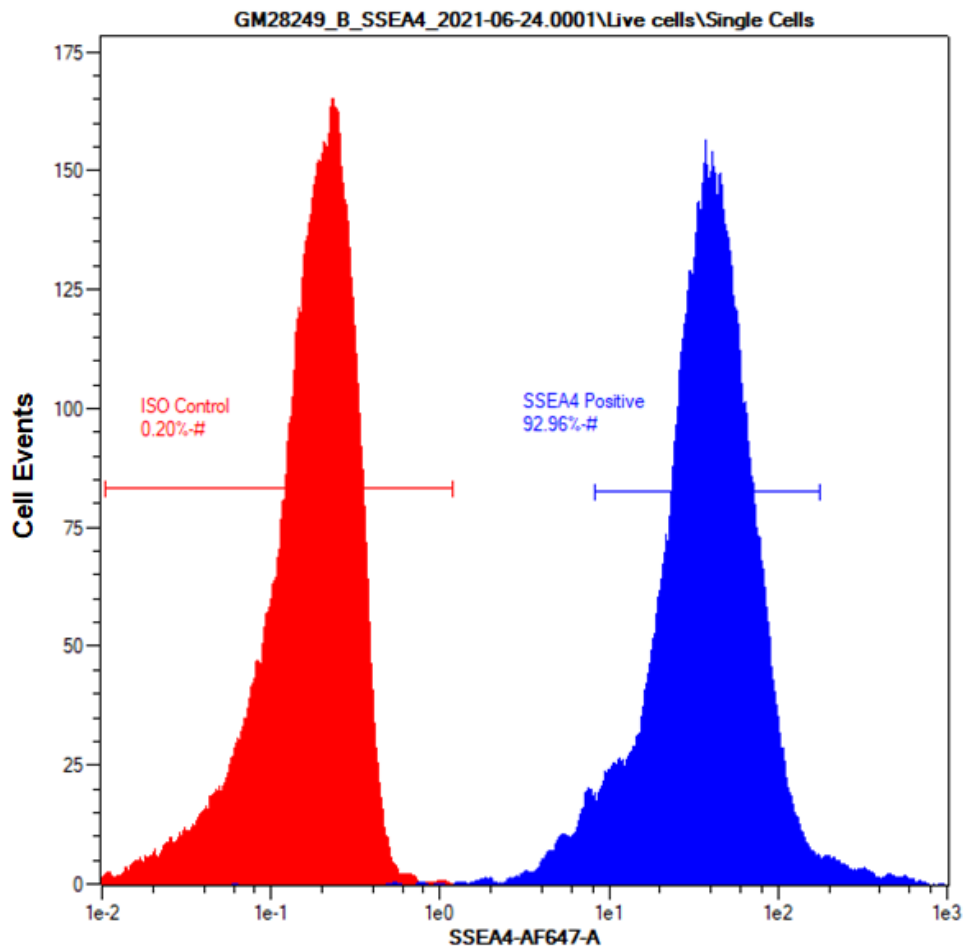


**Figure 2.** iPSC colonies showing alkaline phosphatase activity



## Surface Antigen Expression of Stem Cell Markers

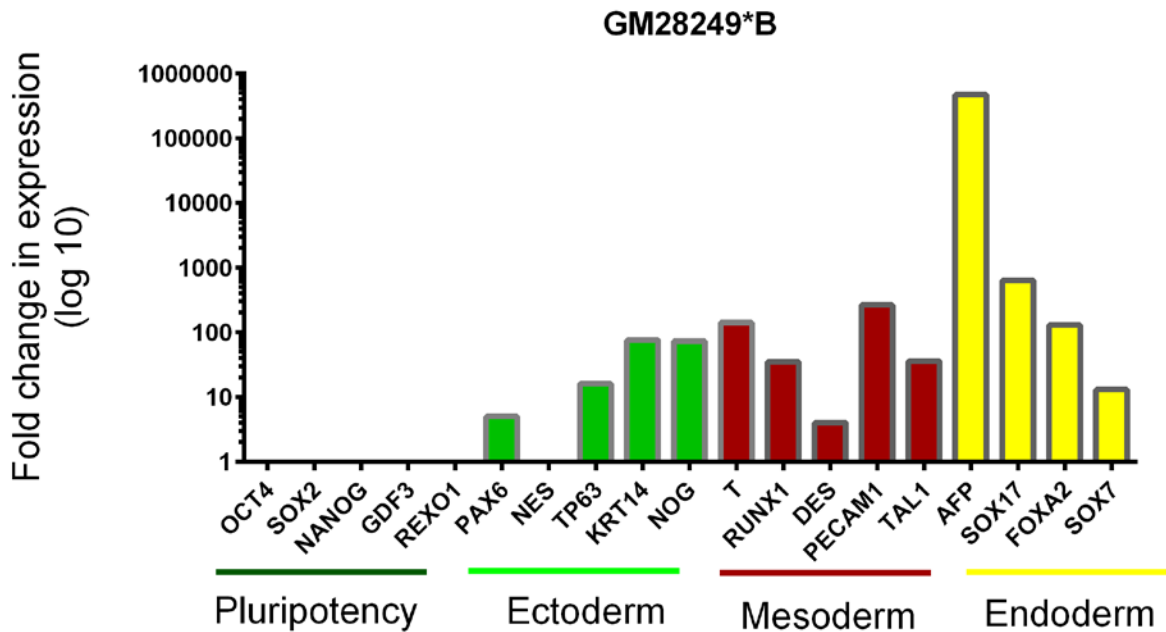
Undifferentiated cells are stained for stage specific embryonic antigen 4 (SSEA4) which is expressed on the surface of undifferentiated human pluripotent stem cells. Cells were analyzed using the MACSQuant Flow Cytometer by Miltenyi Biotec. More than 80% of cells should stain with antibodies specific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



**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	5	T	141	AFP	471785
SOX2	0	NES	0	RUNX1	35	SOX17	637
NANOG	0	TP63	16	DES	4	FOXA2	129
GDF3	0	KRT14	76	PECAM1	265	SOX7	13
REXO1	1	NOG	73	TAL1	36		

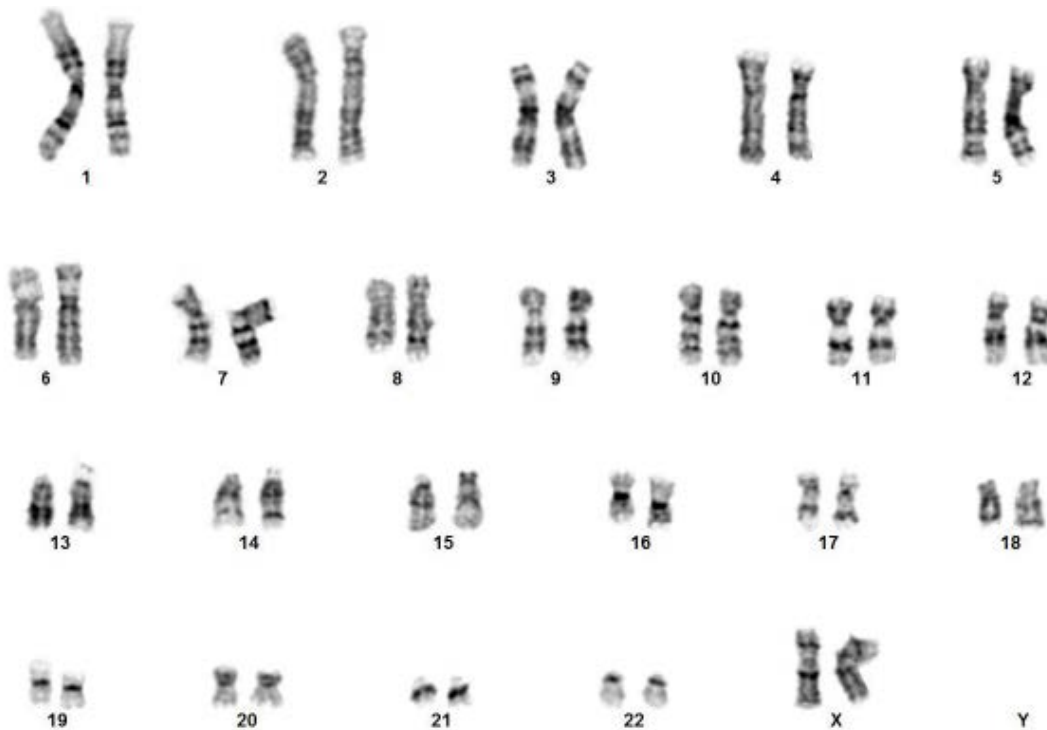
**Figure 4.** Fold change in expression of pluripotency genes and tri-lineage specific genes

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

Cytogenetic Banding Technique	G-banding
Passage at Analysis	P17
Metaphase Cells Counted	20
Metaphase Cells Analyzed	5
Metaphase Cells Karyotyped	5
Short ISCN	46, XX [20]



**Figure 5.** G-banding karyogram