



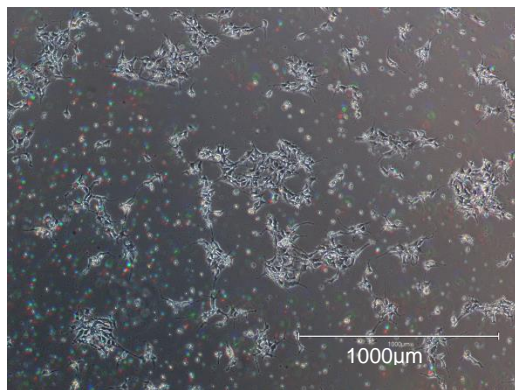


## Post-Thaw Cell Viability

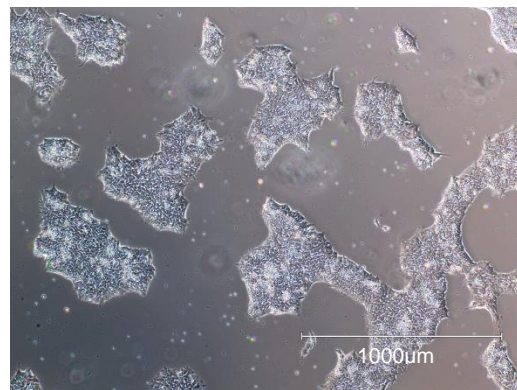
One distribution lot vial of the cell line was thawed and placed in culture. Cultures were observed daily. Colonies were photographed upon first appearance, then 2 days later. Colonies must double in diameter within 5 days. The area for 5 colonies was measured using image analysis software. The average area is reported here.

Day	Average area ( $\mu\text{m}^2$ )
1	6872
3	42664

Colony area increased by 6 fold.



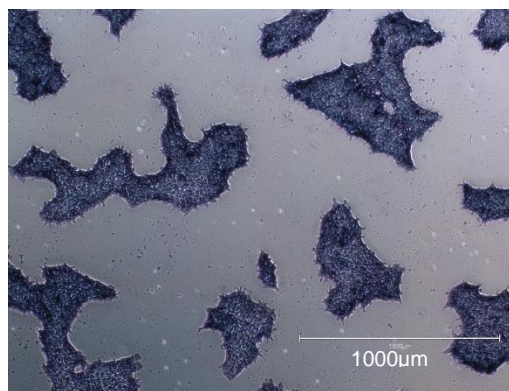
**Figure 1A.** Colonies post thaw (Day 1)



**Figure 1B.** Colonies 2 days after first observation (Day 3)

## Alkaline Phosphatase Staining

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

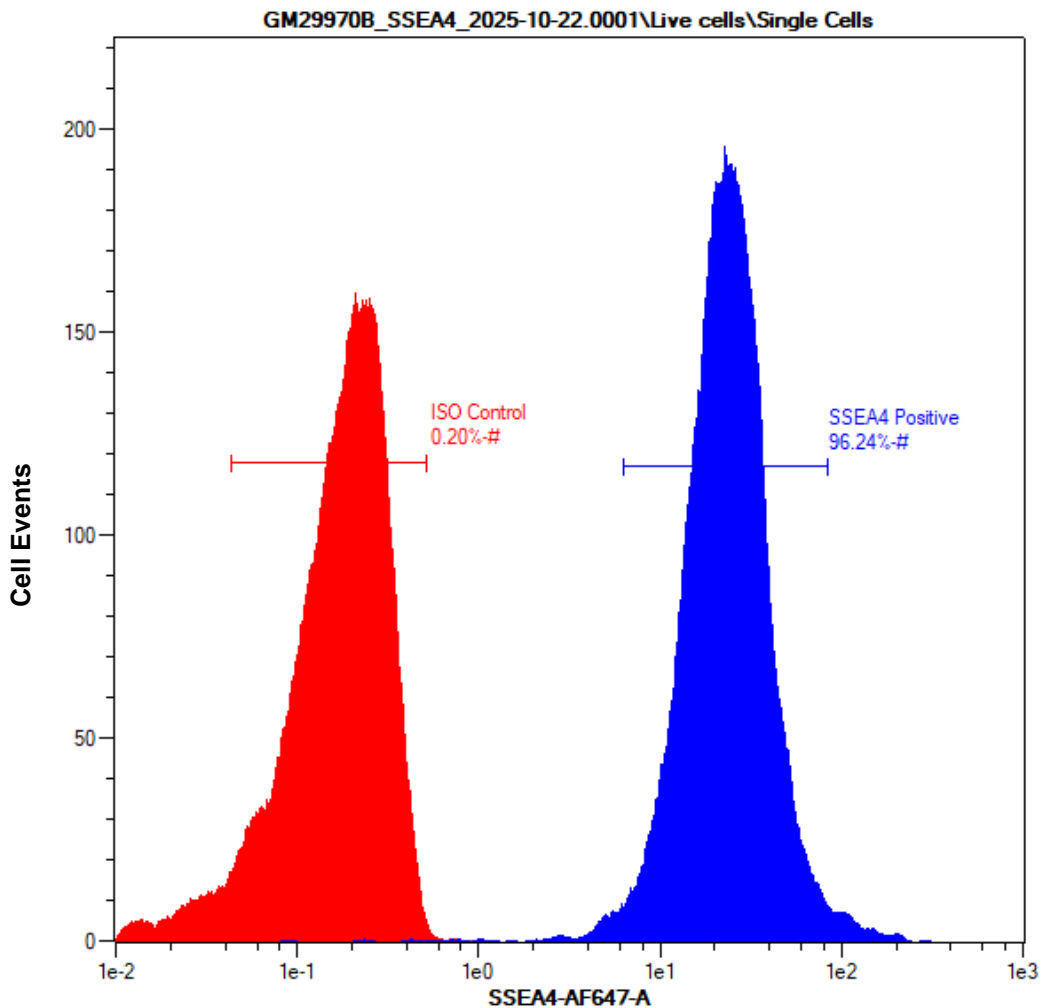


**Figure 2.** iPSC colonies showing alkaline phosphatase activity



## 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.

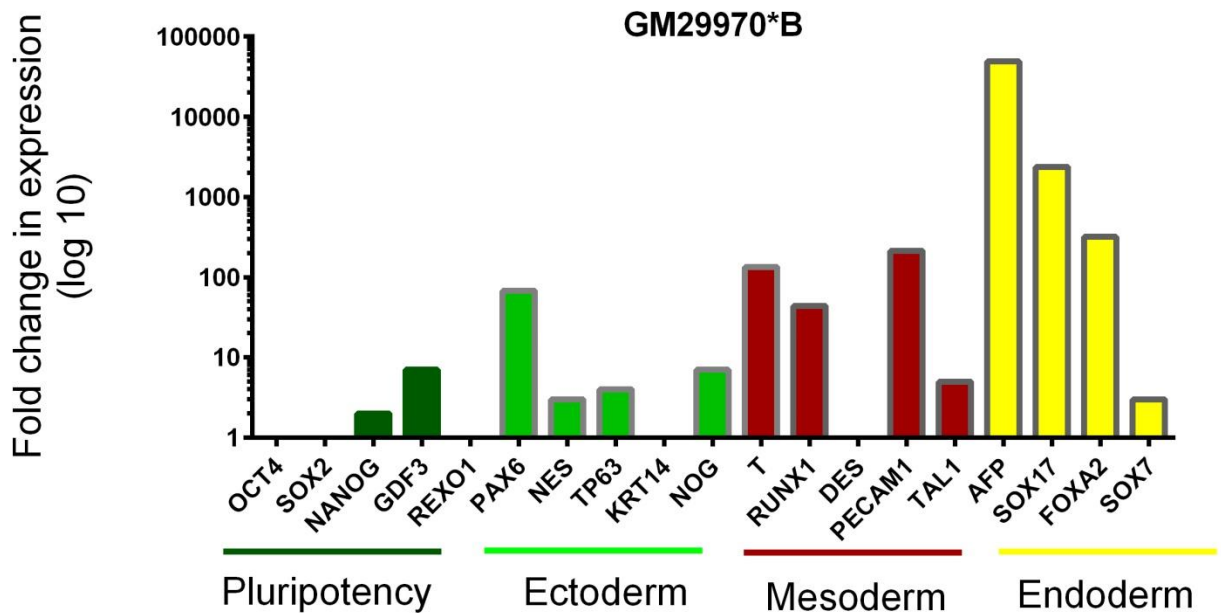


**Figure 3.** Representative histogram of SSEA4 positive population showing an overlay of isotype stained control (red) and SSEA4 positive population (blue)



## 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	68	T	134	AFP	49084
SOX2	0	NES	3	RUNX1	44	SOX17	2376
NANOG	2	TP63	4	DES	1	FOXA2	318
GDF3	7	KRT14	0	PECAM1	214	SOX7	3
REXO1	0	NOG	7	TAL1	5		

**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)



## Cytogenetics

Cytogenetic Banding Technique	G-banding
Passage at Analysis	P14
Metaphase Cells Counted	20
Metaphase Cells Analyzed	5
Metaphase Cells Karyotyped	5
Short ISCN	46,XY[20]



**Figure 5.** G-banding karyogram