



Stem Cell Research

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iPS Cell Reprogramming

Reprogramming of adult cells into induced pluripotent stem cells (iPS) has provided an important new vehicle to facilitate stem cell research. Recent studies have shown that this may be accomplished by the introduction of key genes into somatic cells by transduction with various viral vectors or transfection of plasmids.

Retroviral and lentiviral vectors appear to achieve among the highest levels of efficiency of iPS cell generation. We offer an extensive collection of vectors for iPS cell reprogramming.

Retroviral Vectors and Packaging Cells for iPS Cell Generation

Our iPS retroviral vectors are constructed from the pMXs vector backbone developed by Dr. Toshio Kitamura at the University of Tokyo.* Each vector contains one of 6 factors shown to help reprogram adult fibroblasts into iPS cells. Both human and mouse genes are available individually or in sets. Separate retroviral vectors are available for p53 shRNA, which has been shown to potentially increase the efficiency of iPS cell generation.

Platinum Retroviral Packaging Cells provide an easy way to produce high-titer retroviruses from these stem cell plasmids. For additional information on these cell lines please see [p. 63](#).

*Kitamura, T. et al. (2003). *Exp. Hematol.* 31:1007-1014.

Human iPS Vectors

Target Name	Vector Backbone	Catalog Number
Oct-3/4	pMXs	RTV-701
Sox2	pMXs	RTV-702
c-Myc	pMXs	RTV-703
Klf4	pMXs	RTV-704
NANOG	pMXs	RTV-709
Lin28	pMXs	RTV-710
Set of 4 vectors (Oct-3-4, Sox2, c-Myc, Klf4)	pMXs	RTV-701-C
Set of 6 vectors (Oct-3-4, Sox2, c-Myc, Klf4, NANOG, Lin28)	pMXs	RTV-709-C
p53 shRNA	pRetro	RTV-410

Mouse iPS Vectors

Target Name	Vector Backbone	Catalog Number
Oct-3/4	pMXs	RTV-705
Sox2	pMXs	RTV-706
c-Myc	pMXs	RTV-707
Klf4	pMXs	RTV-708
NANOG	pMXs	RTV-711
Lin28	pMXs	RTV-712
Set of 4 vectors (Oct-3-4, Sox2, c-Myc, Klf4)	pMXs	RTV-705-C
Set of 6 vectors (Oct-3-4, Sox2, c-Myc, Klf4, NANOG, Lin28)	pMXs	RTV-711-C
p53 shRNA	pRetro	RTV-400

Retroviral Packaging Cell Lines

Product Name	Size	Catalog Number
Platinum-E Retroviral Packaging Cell Line, Ecotropic	$\geq 3 \times 10^6$ cells	RV-101
Platinum-A Retroviral Packaging Cell Line, Amphotropic	$\geq 3 \times 10^6$ cells	RV-102
Platinum-GP Retroviral Packaging Cell Line, Pantropic	$\geq 3 \times 10^6$ cells	RV-103
pCMV-VSV-G Packaging Vector (for use with Platinum-GP cells)	10 μ g	RV-110

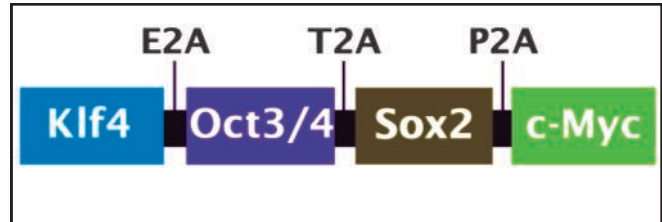
Polycistronic Vectors for iPS Cell Generation

Our Polycistronic Viral Vectors provide a convenient way to generate iPS cells. The defined stem cell factors Klf4, Oct-3/4, Sox2 and c-Myc are in-frame fused into a single open reading frame (ORF) by self-cleaving 2A peptides. The transcription factor ORF is followed by IRES-GFP as a reporter to verify viral transduction into your target cell. Efficiencies of iPS generation are typically higher compared to transduction of four separate viruses each containing a single gene.

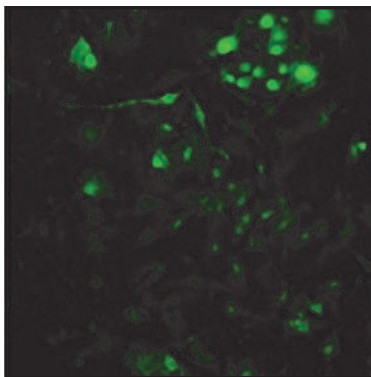
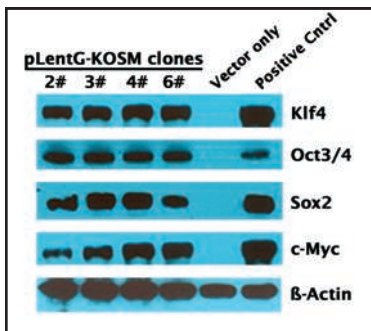
Two vectors are available:

- pLentG-KOSM is a lentiviral vector containing mouse sequences
- pRetroG-OKSM is a retroviral vector containing human sequences

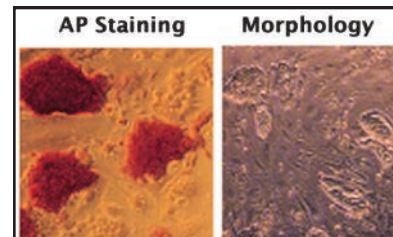
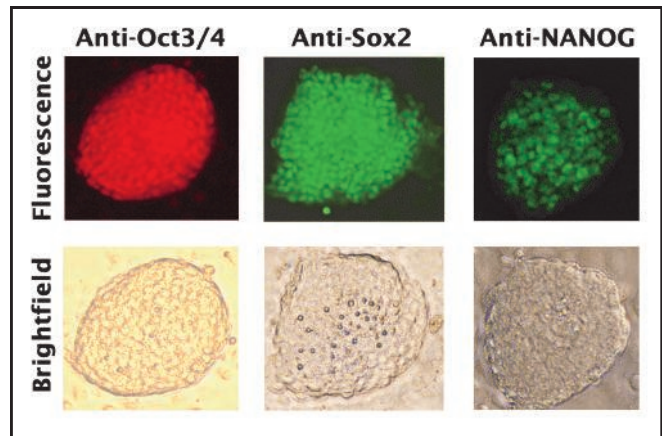
- **More Efficient:** Up to 10-fold higher efficiency compared to multi-virus transduction, and 500-fold compared to non-viral methods
- **Reporter Convenience:** GFP reporter gene helps to monitor viral transduction



Open Reading Frame of pLentG-KOSM Lentiviral Vector.



Expression of Stem Cell Factors and GFP. Top: Transient expression of KOSM fusion gene in 293T cells confirmed by Western blot. Bottom: GFP fluorescence in MEF cells 3 days after infection with lentivirus containing KOSM fusion.



Characterization of iPS Cell Colonies Generated from MEFs Infected with Lentivirus Containing the KOSM Fusion. Top: Staining of pluripotency markers in induced cell colonies at 200x magnification. Bottom: AP staining at 100x magnification and morphology at 40x magnification in induced cell colonies.

Product Name	Size	Catalog Number
pLentG-KOSM Polycistronic Lentiviral Vector (Mouse genes)	100 µL	LTV-700
pRetroG-OKSM Polycistronic Retroviral Vector (Human genes)	100 µL	RTV-700

For efficient packaging of your virus, please see our Lentiviral Packaging Systems on **p. 58** and Retroviral Packaging Cell Lines on **p. 63 and 66**.

Platinum Retroviral Expression Systems for Stem Cells

Retroviral vectors are useful for delivering genes of interest into a host cell where integration into the genome is desired. However, traditional retroviral expression technologies usually result in low viral titers which make gene expression studies difficult.

Our Platinum Retroviral Expression Systems incorporate superior packaging cell lines and vector technologies to produce high-titer virus with a single plasmid transfection. The Platinum Expression Systems include one of our exclusive Platinum Packaging Cell Lines which already contain the gag and pol genes; the Ecotropic and Amphotropic cells also contain an envelope protein. Simply clone your gene of interest into the vector provided and transfect into the Platinum cells. If you choose a Pantropic system, simply co-transfect with the VSV-G plasmid provided.

The Platinum Expression Systems below are specially designed for superior expression with either ES/EC cells or hematopoietic stem cells. For more information on our Platinum Expression Systems for a variety of cells, please see **page 64**.

- **Higher Viral Yields:** Average titer 10^7 infectious units/mL with transient transfection
- **Versatile:** 3 Packaging cell lines for use with nearly any target host species
- **Optimized for Stem Cell Studies:** Specially designed expression systems for ES/EC cells or hematopoietic stem cells

	Amphotropic	Ecotropic	Pantropic
Human	+++	N.S.	+++
Mouse	+++	+++	+++
Rat	+++	+++	+++
Monkey	+++	N.S.	+++
Cat	+++	N.S.	+++
Dog	+++	N.S.	+++
Hamster	+	N.S.	+++
Bird	N.S.	N.S.	+++
Fish	N.S.	N.S.	+++
Frog	N.S.	N.S.	+++
Insect	N.S.	N.S.	+++
Mollusk	N.S.	N.S.	+++

*Virus must be packaged with a pantropic envelope protein such as VSVG.
N.S. = Not Suitable

Suitability of Platinum Retroviral Expression Systems by Host Species.

Recent Product Citation

Kishida, T. et al (2015). Reprogrammed functional brown adipocytes ameliorate insulin resistance and dyslipidemia in diet-induced obesity and type 2 diabetes. *Stem Cell Reports* 10.1016/j.stemcr.2015.08.007. (VPK-303 and VPK-305)

Catalog Number	Packaging Cell Line	Transfer Vector	Envelope Vector	Control Vector
VPK-303	Plat-E (Ecotropic)	pMCs-Puro	————	pMCs-GFP
VPK-304	Plat-A (Amphotropic)	pMCs-Puro	————	pMCs-GFP
VPK-305	Plat-GP (Pantropic)	pMCs-Puro	pCMV-VSV-G	pMCs-GFP
VPK-306	Plat-E (Ecotropic)	pMYs-Puro	————	pMYs-GFP
VPK-307	Plat-A (Amphotropic)	pMYs-Puro	————	pMYs-GFP
VPK-308	Plat-GP (Pantropic)	pMYs-Puro	pCMV-VSV-G	pMYs-GFP

Components of the Platinum Retroviral Expression Systems for Stem Cells.

Product Name	Size	Catalog Number
Platinum ES/EC Retroviral Expression System, Ecotropic	1 kit	VPK-303
Platinum ES/EC Retroviral Expression System, Amphotropic	1 kit	VPK-304
Platinum ES/EC Retroviral Expression System, Pantropic	1 kit	VPK-305
Platinum HSC Retroviral Expression System, Ecotropic	1 kit	VPK-306
Platinum HSC Retroviral Expression System, Amphotropic	1 kit	VPK-307
Platinum HSC Retroviral Expression System, Pantropic	1 kit	VPK-308

Stem Cell Feeders

Leukemia inhibitory factor (LIF) is useful for maintaining the undifferentiated state of mouse embryonic stem (mES) cells. However, LIF does not have the same effect on human embryonic stem (hES) cells. Therefore, hES cells require the use of feeder cells for both derivation and maintenance. We offer a variety of feeder cells for stem cell culture. All feeder cells must be mitotically inactivated prior to use.

SNL 76/7 Passage-Independent Feeder Cells for iPS Culture

The SNL 76/7 is an immortalized cell line derived from mouse fibroblast STO cells which have been transformed with murine LIF and neomycin resistance genes.

- **Superior Culture:** Transformed with LIF gene for better maintenance of undifferentiated state
- **Versatile:** Useful for culture of human and mouse iPS cells and as a feeder for ES cells
- **Passage-Independent:** Immortalized cell line

Recent Product Citations

1. Arai, Y. et al. (2015). Spectral fingerprinting of individual cells visualized by cavity-reflection-enhanced light-absorption microscopy. *PLoS One* **10**:e0125733.
2. Wu, D.T. and Roth, M.J. (2014). MLV based viral-like particles for delivery of toxic proteins and nuclear transcription factors. *Biomaterials* **35**:8416-8426.
3. Takenaka-Ninagawa, N. et al. (2014). Generation of rat-induced pluripotent stem cells from a new model of metabolic syndrome. *PLoS One* **9**:e104462.
4. Pioyan, C. et al. (2014). Generation of mouse lines conditionally over-expressing microRNA using the Rosa26-Lox-Stop-Lox system. *Methods Mol. Biol.* **1194**:2103-224.

Product Name	Size	Catalog Number
SNL Feeder Cells	3 x 10 ⁶ cells	CBA-316

JK1 Passage-Independent Feeder Cells

JK1 is an immortalized CD34+ stromal cell line that supports long-term proliferation of stem cells. It has been shown to maintain capacity for stem cell renewal after serial passaging for over one year. JK1 may be used to culture a variety of cell types including pluripotent ES cells, germ-line derived stem cells, and primordial germ cell-derived EG cells.

Recent Product Citations

1. Burnight, E.R. et al (2014). CEP290 gene transfer rescued Leber congenital amaurosis cellular phenotype. *Gene Ther.* **21**:662-672.
2. Martin, L.A. et al. (2013). Serial enrichment of spermatogonial stem and progenitor cells (SSCs) in culture for derivation of long-term adult mouse SSC lines. *J. Vid. Exp.* **72**:e50017.

Product Name	Size	Catalog Number
JK1 Feeder Cells	1 x 10 ⁶ cells	CBA-315

MEF Feeder Cells

Our murine embryonic fibroblast (MEF) feeder cells are useful for the maintenance of human or mouse ES cells in their undifferentiated state. Cells must be mitotically inactivated prior to use.

Product Name	Size	Catalog Number
MEF Feeder Cells	5 x 10 ⁶ cells	CBA-310
MEF Feeder Cells, Hygromycin-resistant	5 x 10 ⁶ cells	CBA-313
MEF Feeder Cells, Neomycin-resistant	5 x 10 ⁶ cells	CBA-311
MEF Feeder Cells, Puromycin-resistant	5 x 10 ⁶ cells	CBA-312

CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

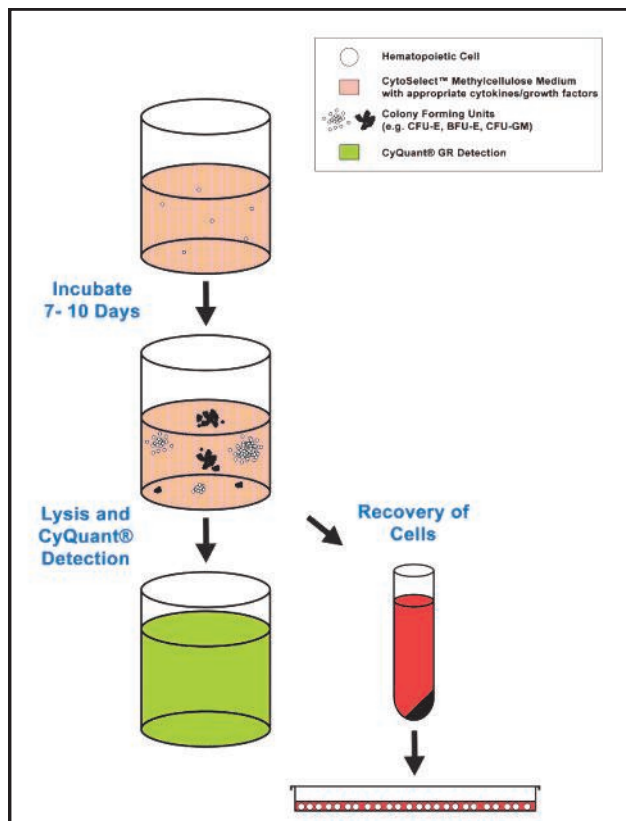
Hematopoietic stem cells (HSCs), when cultured in a suitable semisolid matrix such as methylcellulose supplemented with cytokines & nutrients, proliferate to form discrete cell clusters or colonies. Such HSCs or hematopoietic progenitors are known as colony-forming cells (CFCs). In classic CFC assays, cells are cultured in a 35mm dish for 14-21 days so the colonies can reach a certain size for manual counting, which can be tedious and subjective.

The CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay provides a high-throughput method to quantify CFCs in just 7-10 days with no manual cell counting required. Cells are lysed, solubilized, and quantified using a fluorescent dye included in the kit. Alternatively, cells may be recovered for further culture and analysis.

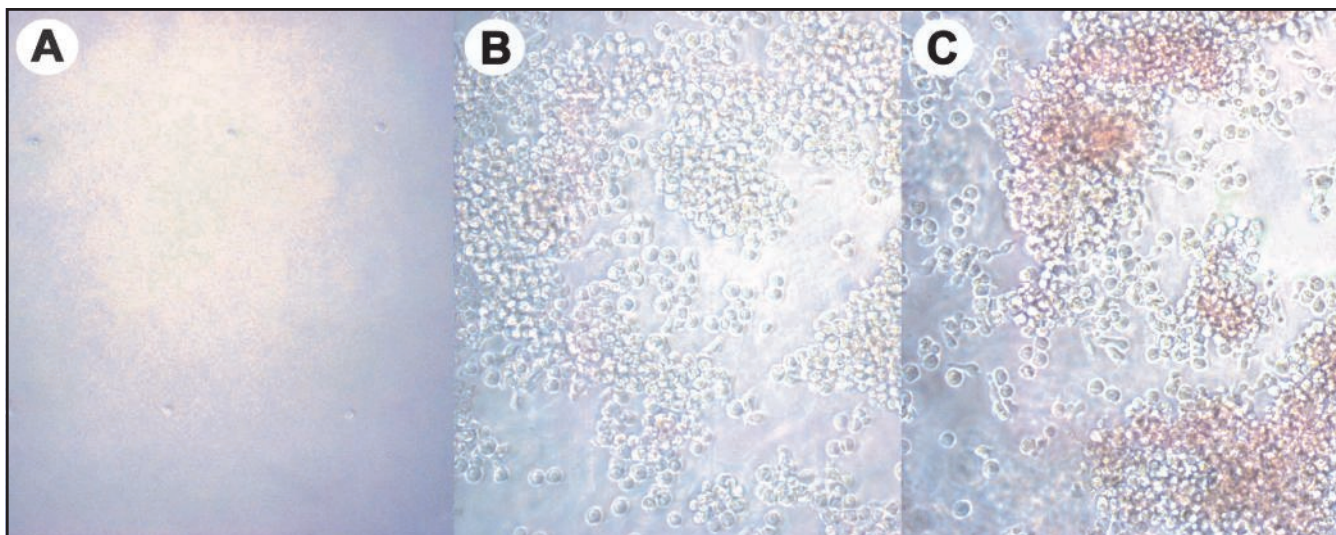
- **Fast Results:** 7-10 days vs. 2-3 weeks
- **Convenient:** Eliminates manual counting
- **Easier Reagent Handling:** Methylcellulose media can be handled using a pipet instead of a syringe

Recent Product Citations

1. Chiba, H. et al. (2013). Diabetes impairs the interactions between long-term hematopoietic stem cells and osteopontin-positive cells in the endosteal niche of mouse bone marrow. *Am. J. Physiol. Cell Physiol.* **305**:C693-C703.
2. Neri, P. et al. (2011). Bortezomib-induced "BRCAness" sensitizes multiple myeloma cells to PARP inhibitors. *Blood* **118**:6368-6379.



Assay Principle for the CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay.



HSC Colony Formation. Human bone marrow derived CD34+ Hematopoietic Progenitor Cells were seeded at 3000 cells/well and cultured for 7-10 days in the absence or presence of growth factors/cytokines. Colonies were quantified according to the assay protocol. **A:** After 7 days without cytokines. **B:** After 7 days in presence of cytokines. **C:** After 10 days in presence of cytokines (hemoglobin visible).

Product Name	Detection	Size	Catalog Number
CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay	Fluorometric	96 Assays	CBA-320
		5 x 96 Assays	CBA-320-5

StemTAG™ 96-Well Stem Cell Colony Formation Assay

Our StemTAG™ 96-Well Stem Cell Colony Formation Assay provides a high-throughput method to quantify ES cells in just 7-10 days with no manual cell counting required.

After colonies are formed, stem cells may be analyzed in 3 ways:

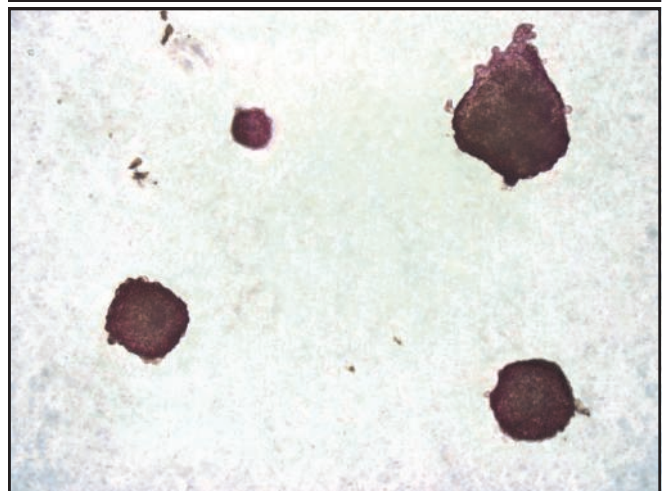
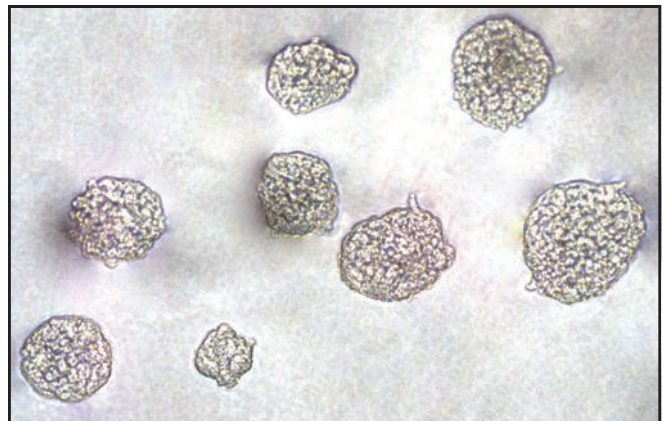
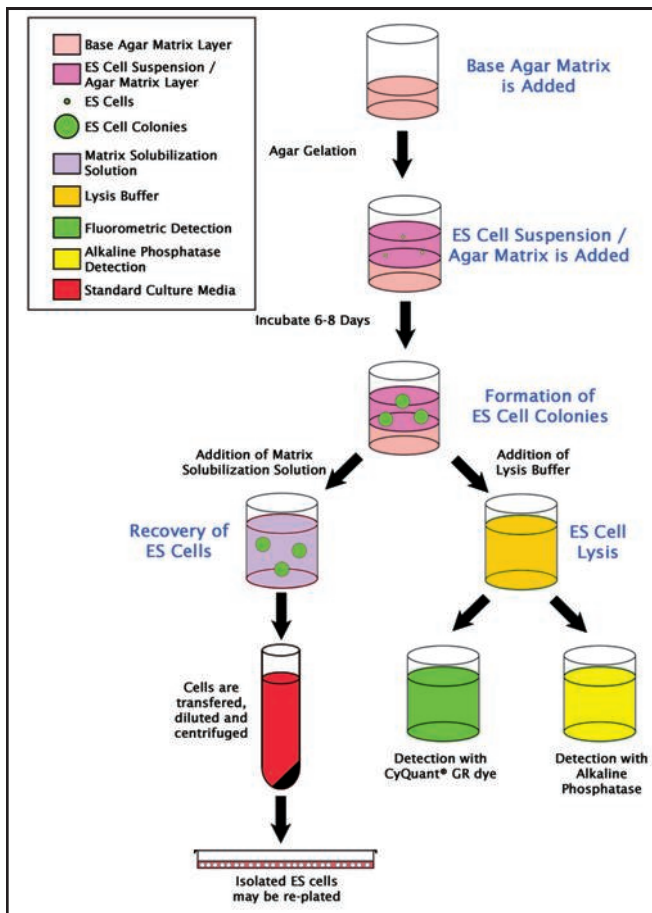
1. Lyse cells, then quantify using a fluorescent dye included in the kit.
2. Lyse cells, then measure alkaline phosphatase activity using reagents provided.
3. Recover colonies for further culture and analysis.

This assay may be of particular interest for the study of tumor stem cells.

- **Fast Results:** 7-10 days vs. 2-3 weeks using conventional methods
- **Versatile:** Quantify cells using fluorescent dye, measure alkaline phosphatase activity, or recover cells for further analysis
- **Plate Reader Convenience:** No manual cell counting required

Recent Product Citation

Shin, M.R. et al (2015). Isocudraxanthone K induces growth inhibition and apoptosis in oral cancer cells via hypoxia inducible factor-1 α . *Biomed. Res. Int.* **2014**:934691.



StemTAG™ Stem Cell Colony Formation Assay Principle.

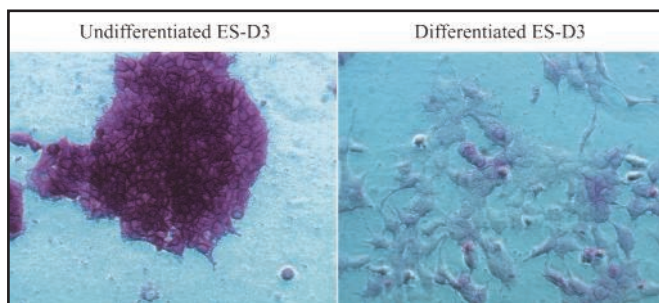
Anchorage-Independent Growth of Mouse ES-D3 Cells.
Top: Phase Contrast. **Bottom:** Alkaline Phosphatase Staining.

Product Name	Detection	Size	Catalog Number
StemTAG™ 96-Well Stem Cell Colony Formation Assay	Fluorometric	96 Assays	CBA-325
		5 x 96 Assays	CBA-325-5

StemTAG™ Alkaline Phosphatase Assay Kits

The StemTAG™ Alkaline Phosphatase Staining and Activity Assay Kits monitor AP activity via both immunocytochemistry staining and a colorimetric 96-well plate-based activity assay. The staining and activity assay kits are also sold separately.

- **Fast Results:** Staining and Activity Assay protocols each take less than 1 hour
- **Versatile:** Useful for human ES, EG and EC cells, as well as mouse ES and EG cells



StemTAG™ Alkaline Phosphatase Staining Kit. Murine embryonic stem cells (ES-D3) were maintained in an undifferentiated state with LIF. To induce differentiation, LIF was withdrawn over several days. Various differentiation events were observed: cells became flattened and enlarged with reduced proliferation. On day 5, cells were stained according to the assay protocol.

Recent Product Citations

1. Lee, K.H. et al. (2015). Subculture of germ cell-derived colonies with GATA4-positive feeder cells from neonatal pig testes. *Stem Cells Int.* 6029271. (CBA-300)
2. Jacinto, F.V. et al. (2015). The nucleoporin Nup153 regulates embryonic stem cell pluripotency through gene silencing. *Genes Dev.* 29:1224-1238. (CBA-300)
3. Langlois, T. et al. (2014). TET2 deficiency inhibits mesoderm and hematopoietic differentiation in human embryonic stem cells. *Stem Cells* 32:2084-2097. (CBA-300)
4. Lee, K.H. et al. (2014). Identification and in vitro derivation of spermatogonia in beagle testis. *PLoS One* 9:e109963. (CBA-300)
5. Manukyan, M. and Singh, P.B. (2014). Epigenome rejuvenation: HP18 mobility as a measure of pluripotent and senescent chromatin ground states. *Sci. Rep.* 4:4789. (CBA-300)
6. Yue, Y. et al. (2015). Safe and bodywide muscle transduction in young adult Duchenne muscular dystrophy dogs with adeno-associated virus. *Hum. Mol. Genet.* 10.1093/hmg/ddv310. (CBA-310)
7. Pino-Barrio, M.J. et al. (2015). V-myc immortalizes human neural stem cells in the absence of pluripotency-associated traits. *PLoS One* 10:e0118499. (CBA-301)
8. Pan, X. et al. (2015). AAV-8 is more efficient than AAV-9 in transducing neonatal dog heart. *Hum. Gene Ther. Methods* 10.1089/hgtb.2014.128. (CBA-301)
9. Dong, Y. et al. (2014). NOTCH-mediated maintenance and expansion of human bone marrow stromal/stem cells: a technology designed for orthopedic regenerative medicine. *Stem Cells Trans. Med.* 3:1456-1466. (CBA-301)
10. Dixon, J.E. et al. (2014). Combined hydrogels that switch human pluripotent stem cells from self-renewal to differentiation. *PNAS* 111:5580-5585. (CBA-301)
11. Guo, L. et al. (2014). Effects of erythropoietin on osteoblast proliferation and function. *Clin. Exp. Med.* 14:69-76. (CBA-301)

Product Name	Detection	Size	Catalog Number
StemTAG™ Alkaline Phosphatase Staining and Activity Assay Kit	ICC & Colorimetric	2 x 100 Assays	CBA-302
	ICC & Fluorometric	2 x 100 Assays	CBA-308
StemTAG™ Alkaline Phosphatase Activity Assay Kit	Colorimetric	100 Assays	CBA-301
	Fluorometric	100 Assays	CBA-307
StemTAG™ Alkaline Phosphatase Staining Kit (Purple)	ICC	100 Assays	CBA-306
StemTAG™ Alkaline Phosphatase Staining Kit (Red)	ICC	100 Assays	CBA-300

StemTAG™ PCR Primer Set for Stem Cell Characterization

Pluripotent stem cells can differentiate into cells derived from all three embryonic germ layers: endoderm, mesoderm and ectoderm. Our StemTAG™ PCR Primer Set provides an efficient system for monitoring ES cell differentiation/undifferentiation. Seven primer sets are included: primers for two widely studied stem cell markers (Oct-4 and NANOG), one marker for each embryonic germ layer (AFP/Endoderm, Flk-1/Mesoderm and NCAM/Ectoderm), and two controls (GAPDH and β -Actin). Primers are suitable for either end-point or real-time (quantitative) PCR.

Product Name	Size	Catalog Number
StemTAG™ PCR Primer Set for Stem Cell Characterization	50 Reactions	CBA-303



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