

ANH Application Catalog

Induced Pluripotent Stem Cells (iPSC) Reprogramming

Induced Pluripotent Stem Cells (iPSC) Reprogramming kit

REPROCELL's portfolio includes an extensive product catalog to support stem cell research and 3D bioengineered tissues, a commercial biorepository of ethically-sourced human tissues, extensive clinical networks across the US and Europe that enable our contract research services in human *ex vivo* living tissues, and our capability to supply induced pluripotent stem cells (iPSCs) for both research and clinical applications

REPROCELL's Reprogramming Kits, the StemRNA 3rd Gen Reprogramming Kit and the StemRNA SR Reprogramming Kit are the latest examples of the Stemgent RNA Reprogramming Portfolio. The StemRNA 3rd Gen Reprogramming Kit is the first RNA reprogramming technology to support reprogramming of cells from skin (Fibroblasts), blood (EPCs) and urine (UPCS). The StemRNA SR Reprogramming Kit supports reprogramming EPCs from blood with a single pair of transfections.

ANH provides many products for supporting iPSC Reprogramming starting from RNA reprogramming kits, transfection reagents, T-flask, media, reagents, supplements , and cell substrate for coating plate. For cryopreservation, there are animal component-free, xeno-free, serum-free Cryopreservation Media, cryogenic vials, cryobox, and Coolcell (freezing container). Also, ANH provides LSE (Life Science Equipment) such as liquid handling equipment and compact centrifuge for enhancing workflow efficiency and advancing your research.

REPROCELL's Reprogramming



The StemRNA 3rd Gen Reprogramming Technology is a non-integrating system that uses RNA only to reprogram somatic cells into iPSCs. The Reprogramming Kit contains six reprogramming RNAs (OCT4, SOX2, KLF4, c-MYC, NANOG, LIN28), three immune ablating RNAs and a proprietary miRNA cocktail. Using our footprint-free technology all RNA is cleared from the cells and degraded 18-24h after delivery into the somatic cell.

Key Benefits

Flexible technology generates high-quality human iPS cell lines from multiple target cell types

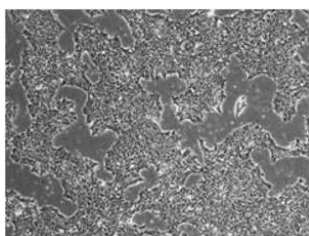
Out-of-the-box reprogramming of cells from skin (fibroblasts), blood (endothelial progenitor cells; EPCs) and urine (urine-derived progenitor cells; UPCs)

- **High efficiency, non-integrating reprogramming**

StemRNA-3rd Gen requires as few as four additional reagents. Double stranded microRNAs enhance reprogramming efficiency, providing iPS cells with high efficiency (up to 4% from fibroblasts, up to 3% from EPCs, up to 0.5% from UPCs).

- **Time-saving protocol delivers faster results facilitating higher throughput**

Colonies are ready to pick in 10-14 days from fibroblasts and 12-16 days from EPCs or UPCs. No screening needed



iPS cells derived from fibroblasts using the StemRNA-3rd Gen Reprogramming Kit were cultured on iMatrix-511 (Cat. No. NP892-011) in NutriStem hPSC XF Medium (Cat. No. 01-0005) for 7 passages. Magnification: 4x

StemRNA-3 rd Gen Reprogramming Kit (00-0076)			
Feature	Fibroblasts	Urine (UPCs)	Blood (EPCs)
No. wells per kit	9	3	3
No Transfections required	4	6-8	6-8
Days to primary iPS cell colonies	10-14	12-14	12-14
Reprogramming efficiency	2-+4%	0.1-0.5%	0.4-3%
Screening Required	No	No	No
Xeno-compatible protocol	Yes	No	No
GMP-compatible RNA manufacturing protocol	Yes	Yes	Yes

Fibroblast Reprogramming Timeline

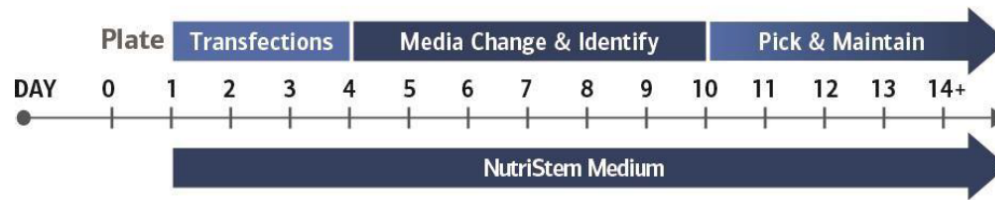


FIGURE 1. Fibroblast Reprogramming Timeline

Day 0: Plate Fibroblasts in Fibroblast Expansion Medium.

Day 1: Media switch to NutriStem Medium.

Day 1-4: Daily NM-RNA cocktail overnight transfections.

Optional on Day 8-14: Identify emerging iPSC colonies by TRA-1-60 live stain.

Day 10-14: Pick primary Fibroblast-NM-RNA-iPSC colonies, replat in NutriStem Medium.

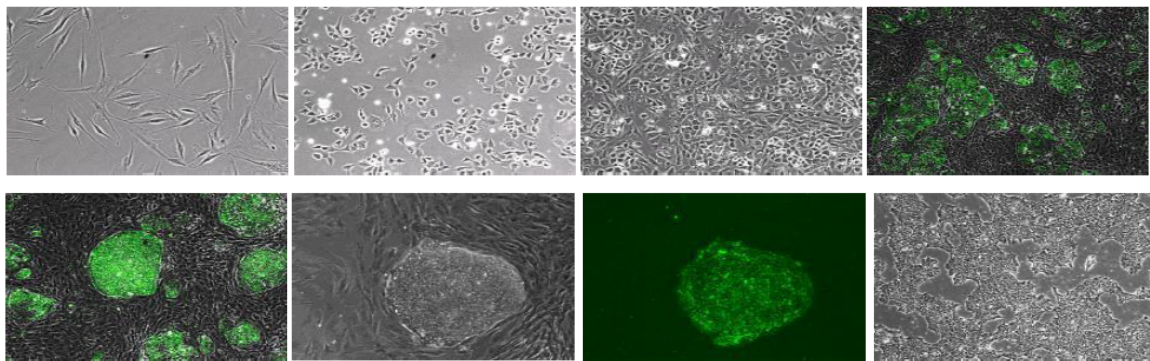


FIGURE 2: Primary reprogramming culture morphology progression, resulting from the reprogramming of adult fibroblasts with the StemRNA 3rd Gen Kit on iMatrix-511 in NutriStem hPSC XF Culture Medium. Day 8, 10 and Day 14 primary Fibroblast-RNA-iPSC colonies were identified using Stemgent StainAlive TRA-1-60 antibody and can be isolated from the primary culture between Day 10-14. Fibroblast-RNA-iPSCs were expanded on iMatrix-511 in NutriStem hPSC XF Culture Medium.

EPC Reprogramming Timeline

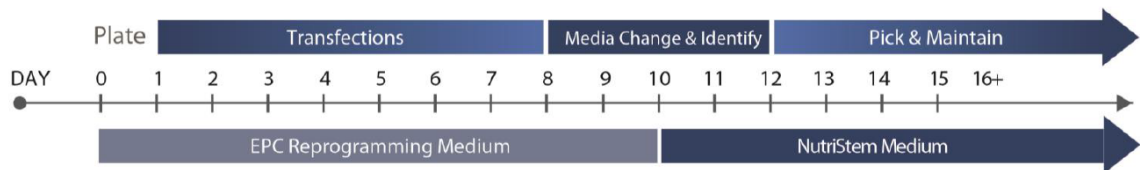


FIGURE 3. EPC Reprogramming Timeline

Day 0: Plate EPCs.

Day 1-8: Daily NM-RNA cocktail transfection overnight.

Day 10: Switch to NutriStem medium. Optional: Identify emerging iPSC colonies by TRA-1-60 live stain

Day 12: Continue culture in NutriStem medium

Day 12-16: Pick primary EPC-NM-RNA-iPSC colonies, replate in NutriStem Medium.

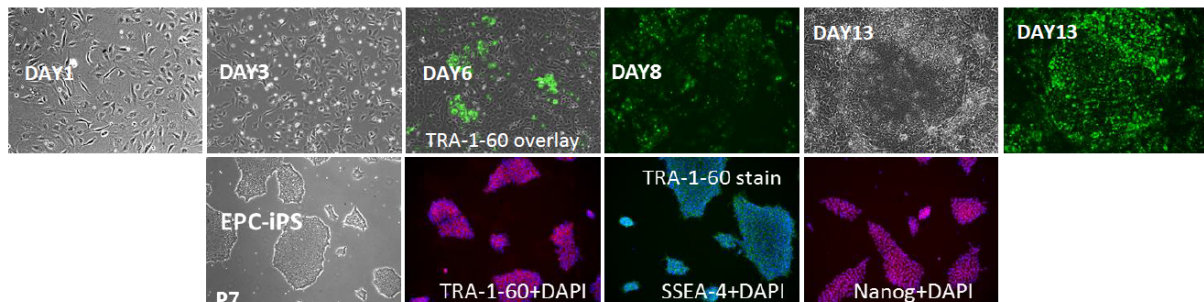


FIGURE 4: Primary reprogramming culture morphology progression resulting from the reprogramming of EPCs with StemRNA 3rd Gen Reprogramming Kit on iMatrix-511 and EPC-Reprogramming Medium containing human serum. Day 6, 8, 13 primary EPC-RNA-iPSC colonies were identified using Stemgent StainAlive TRA-1-60 antibody and can be isolated from the primary culture by Day 12-14. EPC-RNA-iPSCs were expanded on iMatrix-511 in NutriStem XF/FF and stained for pluripotency associated genes at P7 by ICC.

UPC Reprogramming Timeline

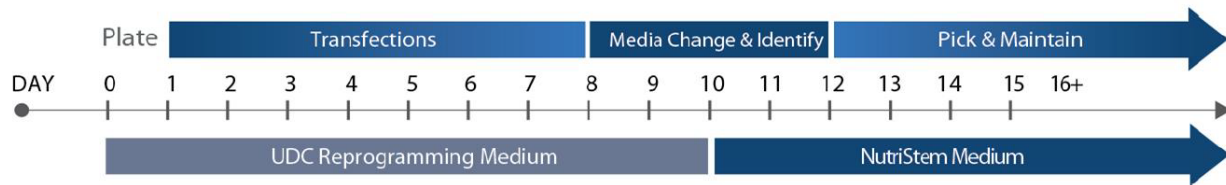


FIGURE 5. UPC Reprogramming Timeline

Day 0: Plate UPCs. Day 1-8: Daily NM-RNA cocktail transfection overnight.

Day 10: Switch to NutriStem Medium.

Optional: Identify emerging iPSC colonies by TRA-1-60 live stain.

Day 12: Continue culture in NutriStem Medium.

Day 12-16: Pick primary UPC-NM-RNA-iPSC colonies, replating in NutriStem Medium.

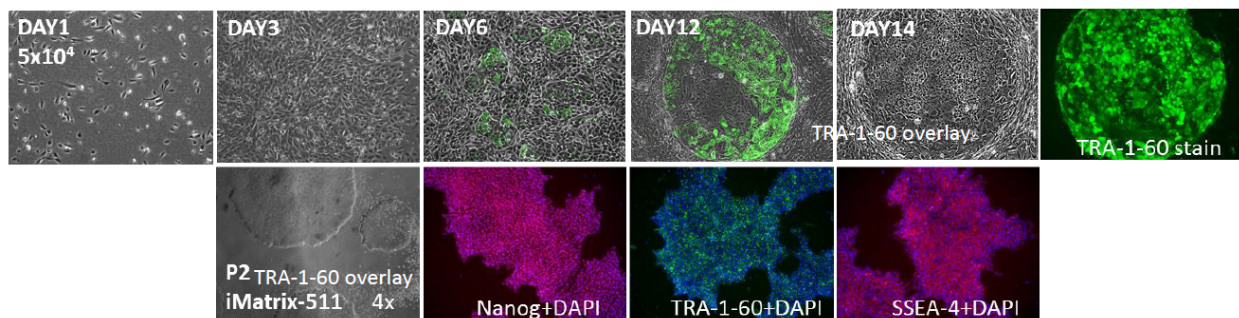


FIGURE 6: Primary reprogramming culture morphology progression, resulting from the reprogramming of UPCs with StemRNA 3rd Gen Kit on iMatrix-511 and UPC-Reprogramming Medium containing human serum. Day 6, 12, 14 primary UPC-RNA-iPSC colonies were identified using Stemgent StainAlive TRA-1-60 antibody and can be isolated from the primary culture by Day 14. UPC-RNA- iPSC were expanded on iMatrix-511 in NutriStem XF/FF and stained for pluripotency associated genes at P7 by ICC.

ANH's Related Products

Brand	Cat. No	Description	Packing
REPROCELL	00-0076	StemRNA 3rd Gen Reprogramming Kit Kit Contents: OKSMNL NM-RNA (Part No. 05-0040), 30 µg, 1 vial EKB NM-RNA (Part No. 05-0041), 22 µg, 1 vial NM-microRNAs (Part No. 05-0042), 15 µg, 1 vial	Kit
REPROCELL	00-0069	Stemfect RNA Transfection Kit (1 kit) Kit contents: RNA Transfection Reagent: 750 µL RNA Transfection Buffer: 30 mL	Kit
REPROCELL	NP892-011	iMatrix 511 Stem Cell Culture Substrate a xeno-free, recombinant Laminin-511 E8	175 µg × 2 tubes
REPROCELL	NP892-012	Fragment produced in CHO-S cells and used with the StemRNA-3rd Gen Reprogramming Kit	175 µg × 6 tubes
REPROCELL	01-2005	NutriStem hPSC XF Medium (500mL) a fully-defined, xeno-free, low growth factor concentration, feeder-free culture medium for human embryonic stem (ES) and induced pluripotent stem (iPS) cells.	500 mL
REPROCELL	01-0020-50	NutriFreez D10 Cryopreservation Medium (previously called CryoStem Freezing Medium) a ready-to-use solution for the animal component-free, xeno-free, serum-free cryopreservation of human embryonic stem (ES), induced pluripotent stem (iPS) and mesenchymal stem cells. The medium contains methylcellulose and DMSO.	50 mL
REPROCELL	03-2002	Stemfactor FGF-basic, Human Recombinant	50 ug
REPROCELL	RCAB004P-F	StemAb™ Anti Human Nanog Antibody Concentration: 0.2 mg/mL	100 µL

REPROCELL	RCAB002P-F	StemAb™ StemAb™ Anti Mouse Nanog Antibody Concentration: 0.2 mg/mL	100 µL
Corning	3516	6 Well Clear, TC Plate w/ Lid, S	50/CS
Corning	3513	12 Well Clear, TC Plate w/ Lid, S	1/Pack, 50/Case
Corning	3524	24 Well Clear, TC Plate, w/ Lid, S	1/Pack, 100/Case
Corning	15-013-CV	500mL DMEM [+] 4.5g/L Glu, Pyr [-] Gln	1 Bot
Corning	15-013-CM	1L DMEM [+] 4.5g/L Glu, Pyr [-] Gln	1 Bot
Corning	25-025-CI	100mL MEM Nonessential Amino Acids, 100X	1 Bot
Corning	35-015-CV	Fetal Bovine Serum, Premium, United States Origin	1 Bot
Corning	25-015-CI	100mL glutaGRO, 200 mM Soln, 100X	1 Bot
Corning	20-031-CV	500mL DPBS, 10X [-] Ca, Mg *C	1 Bot
Corning	25-052-CI	100mL 0.05% Trypsin 0.53 mM EDTA in HBSS	1 Bot
Corning	30-002-CI	100 mL Penicillin-Streptomycin Solution, 100x	1 Bot
Corning	30-003-CF	50 mL Amphotericin B, Liquid	1 Bot
Corning	432021	CoolBox xT, purple, single capacity	1/Case
Corning	432025	CoolBox 2xT, purple, double capacity	1/Case
Corning	432040	CoolRack XT M24, holds 24 x 1.5 or 2ml microfuge Tube,	1/Case
Corning	432049	CoolRack CF15, holds 15 cryovial or FACS Tube	1/Case
Corning	3621	1.7mL Snap Cap Microcentrifuge Tube, PP, NS	500/Pack, 5000/Case
Corning	430639	25cm2 TC Flask, Vent Cap	20/Pack, 200/Case
Corning	4487	5mL Stripette, PS, Paper/Plastic, S	50/Pack, 200/Case
Corning	430791	15mL PP Centrifuge Tube, CentriStar Cap, S	50/Pack, 500/Case
Corning	430829	50mL PP Centrifuge Tube, CentriStar Cap, S	25/Pack, 500/Case

Corning	4110	DW 0.1-10uL Pipet Tip, Natural, NS	1000/Pack, 10000/Case
Corning	4115	DW 0.1-10uL Pipet Tip, Rack, Natural, NS	960/Pack, 3840/Case
Corning	430488	2mL Internal Threaded Cryogenic Vial, SS, S	50/Pack, 500/Case
Corning	432001	CoolCell LX, Cell Freezing Container, for 12 x 1mL or 2mL Cryogenic Vials, Purple	1/Case
Corning	432006	CoolCell FTS30, Freezing Container, for 30 x 1mL or 2mL Cryogenic Vials, Purple	1/Case

StemRNA™ 3rd Gen Reprogramming Kit

00-0076

Brand: **Stemgent™**



The StemRNA 3rd Gen Reprogramming Kit provides the fastest, most efficient method for generating clinically relevant iPS cells using a non-integrating, mRNA-based protocol. This technology supports generating iPS lines derived from fibroblasts, blood, and urine using one multi-purpose kit.

Specifications

Product Name: StemRNA 3rd Gen Reprogramming Kit

Catalog Number: 00-0076

Storage and Stability: Store all three kit components at or below -70°C . Kit components are stable for a minimum of 3 months from date of receipt when stored as directed.

Quality Control: The individual mRNAs are tested for size and integrity. The StemRNA 3rd Gen Reprogramming Kit is functionally validated for successful RNA-based reprogramming of adult fibroblasts, human umbilical vein endothelial cells (HUVECs), EPCs, and UPCs. Complete reprogramming of iPS cell colonies is confirmed by expression of pluripotency markers and appropriate colony morphology. All components of the kit are sterile and have tested negative for *Mycoplasma* spp.

Recommended Usage: For use with associated StemRNA 3rd Gen Reprogramming Kit protocols for generation of iPS cells.

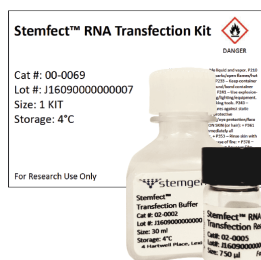
Kit Contents:

- OKSMNL NM-RNA (Part No. 05-0040), 30 μg , 1 vial
- EKB NM-RNA (Part No. 05-0041), 22 μg , 1 vial
- NM-microRNAs (Part No. 05-0042), 15 μg , 1 vial

Stemfect™ RNA Transfection Kit

00-0069

Brand: **Stemgent™**



A proprietary mixture of lipid components is specifically designed for *in vitro* RNA transfection, shown to deliver mRNA, siRNA and miRNA. Provides greater than or equal to 90% transfection efficiency of mRNA into a range of cell types with greater than 95% viability – including human ESCs, Jurkat cells, human fibroblasts, and HEK293T cells.

Stemfect RNA Transfection Kit has been primary fully used to deliver RNA to human ESCs, dendritic cells, and retinal pigment epithelial cells. No need to serum-starve cells using this kit – the transfection efficiency remains greater than or equal to 90% in the presence of serum.

Specifications

Product Name: StemFect RNA Transfection Kit

Catalog Number: 00-0069

Size: 1 Kit

Set Contents:

- RNA Transfection Reagent: 750 µL
- RNA Transfection Buffer: 30 mL

Storage and Stability: RNA Transfection Reagent should be stored at 4 °C and is stable for 6 months when stored as directed. RNA Transfection Reagent is dissolved in ethanol; the vial should be capped immediately after use to minimize evaporation. RNA Transfection Buffer may be stored at –20 °C or 4 °C and is stable for 6 months when stored as directed.

Quality Control: RNA Transfection Reagent has been characterized by mass spectrometry and NMR. StemAb RNA Transfection Kit is functionally tested for transfection efficiency on human fibroblast cells using eGFP mRNA.

iMatrix-511 Stem Cell Culture Substrate

NP892-011 / NP892-012

Brand: **iMatrix™ by Matrixome®**



iMatrix-511 is a xeno-free, recombinant Laminin-511 E8 Fragment produced in CHO-S cells and used with the StemRNA-3rd Gen Reprogramming Kit (00-0076) to generate iPS cells from fibroblasts, blood or urine. iMatrix-511 is a highly purified and refined laminin-511 E8 fragments, produced in CHO cells.

iMatrix-511 features make it an ideal matrix for pluripotent stem cell culture:

- Promotes greater stem cell adhesion than all other matrix proteins that have been tested
- Easy to use (liquid format)
- E8 fragments retain integrin binding specificity and capacity and display higher potency than natural Laminin-511
- Equivalent performance, but lower cost than the legacy iMatrix-511 product

Specifications

Product Name: iMatrix-511 Stem Cell Culture Substrate

Catalog Numbers: NP892-011, NP892-012

Sizes: NP891-011: 2 × 175 µg, NP891-012: 6 × 175 µg

Molecular Weight: 150 kDa

Purity: > 95 % pure

Formulation: Purified Laminin-511 E8 proteolytic fragment

Storage and Stability: Store at 4 °C and protect from light exposure. Stable for 2 years from manufacturing date.

Concentration: 500 µg/mL

Source: CHO cells

Quality Control: Integrin binding $Kd < 10$ nM

Sterility: Sterile

Recommended Usage: iMatrix-511 is suitable for use as a substrate for culture of various cell types, including ES/iPS cells.

NutriStem™ hPSC XF Culture Medium for Human iPS and ES Cells

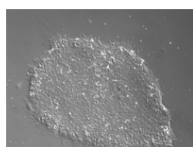
01-0005

Brand: **NutriStem™ by Biological Industries®**

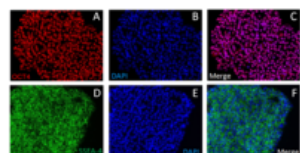


NutriStem hPSC XF Culture Medium is a fully-defined, xeno-free, low growth factor concentration, feeder-free culture medium for human embryonic stem (ES) and induced pluripotent stem (iPS) cells. Cells can be cultured for at least 20 passages while retaining pluripotency marker expression, robust proliferation with a normal karyotype, and the ability to differentiate into cells of all three germ layers *in vitro* and *in vivo*.

- Xeno-free, feeder-free conditions contain no animal components
- Easy, one-step transition from feeder-dependent culture, no adaptation required
- Maintains pluripotency, normal morphology, karyotype, and differentiation potential of human ES and iPS cells over long term culture
- Robust attachment and high cloning efficiency from single cells
- Amenable to a weekend-free culture



Morphology of Human H1 ES cells Grown in NutriStem hPSC XF Medium for 20 passages.



Expression of Pluripotency Markers in hES Cells Cultured in NutriStem hPSC XF medium.

Specifications

Product Name: NutriStem hPSC XF Medium

Catalog Number: 01-0005 (Biological Industries Cat # 05-100-1A): 500 mL

Storage and Stability: Store at -20°C . This product is stable for a minimum of 3 months when stored as directed.

Quality Control: NutriStem Medium tested negative for *mycoplasma*. Human pluripotent stem cells expressed the pluripotency markers SSEA-4, TRA-1-60, and TRA-1-81 after three continuous passages in NutriStem Medium

Sterility: Sterile

NutriFreez™ D10 Cryopreservation Medium

01-0020-50

Brand: **NutriFreez™ by Biological Industries®**



NutriFreez D10 Cryopreservation Medium (previously called CryoStem Freezing Medium) is a ready-to-use solution for the animal component-free, xeno-free, serum-free cryopreservation of human embryonic stem (ES), induced pluripotent stem (iPS) and mesenchymal stem cells. The medium contains methylcellulose and DMSO.

Specifications

Product Name: NutriFreez D10 Cryopreservation Medium

Catalog Number: 01-0020-50 (REPROCELL)

Size: 50 mL

Storage and Stability: Store NutriFreez D10 Cryopreservation Medium at 2-8 °C. Medium is stable until the expiration date listed on the bottle if stored as indicated.

Quality Control:

pH: 7.2-7.6

Appearance: Clear Solution

Performance freezing of hPSC, hMSC, VERO: Pass test

Sterility: Sterile

Stemfactor™ FGF-basic, Human Recombinant

03-0002

Brand: **Stemgent™**



Fibroblast Growth Factor-basic (a.k.a. FGF-basic, FGF-2 or bFGF) plays a central role during development and growth or regeneration of a variety of tissues by promoting cell differentiation and proliferation.

Specifications

Product Name: Stemfactor FGF-basic, Human Recombinant

Catalog Number: 03-0002

Size: 50 µg

Purity: Greater than 95% by SDS-PAGE analysis

Formulation: Lyophilized from 5 mM Tris-HCl, pH 7.5 with 150 mM NaCl.

Reconstitution: Centrifuge briefly and then reconstitute bFGF in 500 µL of 10 mM Tris, pH 7.6, to yield a stock solution of 0.1 mg/mL of bFGF. Avoid freeze-thaw cycles as it can result in loss of activity.

Storage and Stability: Stemfactor bFGF is shipped at room temperature. Lyophilized bFGF is stable for up to 6 months from date of receipt when stored at –20 °C to –80 °C. Reconstituted bFGF, at concentrations greater than or equal to 0.1 mg/mL, is stable for up to 3 months when stored at –20 °C and up to 6 months when stored at –80 °C.

Sterility: Tested to be negative for *Mycoplasmasp.* by PCR and microbial contamination by a sterility test.

Source: *E. coli*

Amino Acid Sequence: MAAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF
LRIHPDGRVD GVREKSDPHI KLQLQAEERG VVSIKVCAN RYLAMKEDGR LLASKCVTDE
CFFFERLESN NYNTYRSRKY TSWYVALKRT GQYKLGSKTG PGQKAILFLP MSAKS

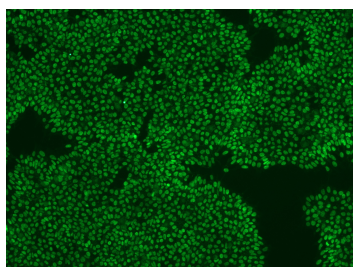
Endotoxin Level: Less than 1.0 EU/µg of bFGF as determined by the LAL method.

Biologic Activity: The ED50 is 0.1 to 1 ng/mL as determined by the dose dependent proliferation of NIH 3T3 cells.

StemAb™ Anti Human Nanog Antibody

RCAB004P-F

Brand: **REPROCELL®**



Transcriptional factors, OCT3/4 (POU5F1) and STAT3 function as key regulators in maintaining pluripotency of stem cells. Thus, POU5F1 and STAT3 have been widely used as molecular markers of pluripotent stem cells. Pluripotential cell-specific Nanog gene is a newly identified homeodomain-bearing transcriptional factor. Importantly, Nanog is expressed specific to early embryos and pluripotent stem cells including mouse and human embryonic stem (ES) and embryonic germ (EG) cells. It is a key molecule involved in the signaling pathway for maintaining the capacity for self-renewal and pluripotency, bypassing regulation by the STAT3 pathway. Therefore, Nanog is one of the molecular markers suitable for recognizing the undifferentiated state of stem cells in the mouse and human.

Specifications

Product Name: StemAb Anti-Human Nanog Antibody

Catalog Number: RCAB004P-F

Purity: Immunogen affinity purified

Storage and Stability: Store at 4 °C (short term), -20 °C or -80 °C (long term), stable for 3 years when stored as directed.

Quality Control:

- Host: Rabbit
- Immunogen: Human Nanog peptide
- Clonality: Polyclonal
- Purity: Immunogen affinity purified
- Concentration: 0.2 mg/mL
- Storage Buffer: PBS (incl. 0.1% sodium azide)

Concentration: 0.2 mg/mL

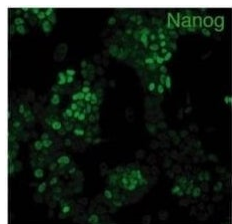
Immunogen: Human Nanog peptide

Clone: Polyclonal

StemAb™ Anti Mouse Nanog Antibody

RCAB002P-F

Brand: **REPROCELL®**



Pluripotential cell-specific Nanog gene is a newly identified homeodomain-bearing transcriptional factor. Importantly, Nanog is expressed specific to early embryos and pluripotent stem cells including mouse and human embryonic stem (ES) and embryonic germ (EG) cells. It is a key molecule involved in the signaling pathway for maintaining the capacity for self-renewal and

pluripotency, bypassing regulation by the STAT3 pathway. Therefore, Nanog is one of the molecular markers suitable for recognizing the undifferentiated state of stem cells in the mouse and human.

Specifications

Product Name: StemAb Anti-Mouse Nanog Antibody

Catalog Number: RCAB001P2P

Purity: Immunogen affinity purified

Storage and Stability: Store at 4 °C (short term), -20 °C or -80 °C (long term), stable for 3 years when stored as directed.

Quality Control

- Host: Rabbit
- Immunogen: Mouse Nanog peptide
- Clonality: Polyclonal
- Purity: Immunogen affinity purified
- Concentration: 0.2 mg/mL
- Storage Buffer: PBS (incl. 0.1% sodium azide)

Concentration: 0.2 mg/mL

Immunogen: Mouse Nanog peptide

Clone: Polyclonal

Find out more at <https://www.anhsci.com/>

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