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# Stem Cell Culture Media and Recombinant Proteins





As cell therapy development continues, the demand for clinically applicable ancillary materials, such as cell culture media and growth factors, is rapidly increasing for industrial use. In particular, stem cell culture media that are used in the manufacture of cell therapy products are required to have higher quality standards than conventional research reagents. This includes consistency between lots, and compliance with various laws and regulations.

StemFit is a brand of stem cell culture media for use from the laboratory to GMP compliant manufacturing of cell therapy products. StemFit was originally developed in collaboration with Prof. Shinya Yamanaka (CiRA, Kyoto Univ.), a Nobel laureate for the discovery of induced pluripotent stem cell (iPSC), to meet the requirements of a clinically relevant human pluripotent stem cell (hPSC) culture media (Sci.Rep. 2014 Jan 8;4:3594). Now, StemFit media is widely used in clinical projects, as well as basic research, around the world.

Importantly, StemFit media for hPSC is designed and manufactured free from animal or human derived components, which ensures high safety and lot-to-lot consistency. Additionally, its well-optimized formulation enables unmatched cell expansion with a flexible weekend-free feeding schedule. Furthermore, its optional one-bottle form (Basic04CT) is a perfect match for advanced manufacturing because it enables the omission of the time-consuming preparation process.





**One Bottle** 

Composition

1

## » StemFit hPSC expansion media





StemFit Basic03

StemFit Basic03 GMP

### Animal-Origin Free & regulatroy compliance for CGT product manufacturing

Animal or human derived components, such as serum derived albumin, or animal cell derived recombinant proteins, are known to carry a risk of hazardous viral contamination for cell therapy. Therefore, StemFit is designed and manufactured under a strict animal origin free policy and is free from animal or human derived components, as referred in USP<1043> and ISO20399. This makes StemFit Basic03 a gold-standard hPSC expansion media for clinical research. To this end, the Japanese PMDA was consulted on the formulation and manufacturing process and found that Basic03 satisfies all requirement of ancillary materials for cell and gene therapy (CGT) manufacturing. Additionally, StemFit offers a GMP compliant product, Basic03 GMP, which is manufactured under applicable GMP guidelines and is thus preferable for CGT products.

### Superior performance in single-cell expansion

Single-cell expansion is an ideal method for CGT product manufacturing since it allows well-controlled, reproducible, and efficient cell expansion. StemFit is optimized for the single-cell expansion method and shows higher cell survival rate in single-cell conditions, which enables efficient and genetically stable cell growth without karyotypic abnormalities.

CGT Catapult, an independent center of excellence to advance the growth of the UK CGT industry, performed a comprehensive comparison program for hPSC culture media and found that StemFit Basic03 to have the highest performance in clinical hPSC expansion.

#### » Figure 1. Easy expansion

(A) Cumulative Population Doublings (CPD)



(B) Average Population Doublings (PD) throughout 5 passages



#### » Figure 2. Consistent gene expression profile

Gene expression data profiled employing the TaqMan<sub>®</sub> ScoreCard<sup>™</sup> assay (n=3)



#### Low expression

**High expression** 

#### » Table 2. Result of karyotyping (CGH array) analysis after expansion

	AK03N	M2	M3	M4	M5
Bank	Normal	Normal	Normal	Normal	Normal
+P9/P10	Normal	Normal	Abnormal	Normal	Normal

Full data is available on the webpage

Web site

### Weekend-Free Feeding

Traditional culture medium requires daily medium changes to maintain hPSCs, which increases the labor cost and risk of human error or contamination in CGT product manufacturing. StemFit allows flexible weekend-free feeding in which hPSC can be expanded healthily without medium change during the weekends. This feature enables researchers to simplify their culture protocol and minimize the labor and culture media costs.



### One-bottle formulation (Only Basic04CT)

Aseptic process in the clean bench or isolator should be simplified to avoid bacterial contamination. StemFit Basic04 Complete Type (Basic04CT) is our next generation hPSC expansion media which has the same features as Basic03 but is delivered in 1 ready-to-use bottle. This feature allows the omission of the mixing process in media preparation which minimizes the risk of bacterial contamination.

#### Large scale production

Large scale cell production for CGT requires a stable supply of large-volume, high-quality ancillary materials.StemFit products are manufactured on a large enough scale to accommodate industrial cell production and have sufficient supply capacity. This feature minimizes the risk of an unstable supply chain or undesirable frequent lot changes. Additionally, other features such as efficient single-cell expansion, ready-to-use one bottle formulation, and weekend-free feeding, are perfect matches for large scale cell production, as well as process scale-up.



StemFit Basic04 Complete Type

#### Multiple product lineups for different research applications

StemFit provides 3 types of media for hPSC expansion, which allows researchers to choose the best solution based on the purpose of their research. For basic or early clinical research, Basic04CT can be the best option since it provides ready-to-use formulation. For clinical projects, Basic03 or Basic03 GMP may be the best option thanks to their unrivaled performance, in addition to GMP regulatory compliance.

#### » Product comparison table

	Basic03	Basic03 GMP	Basic04 Complete Type
Animal origin-free	$\checkmark$	$\checkmark$	$\checkmark$
GMP manufacturing		$\checkmark$	In preparation
bFGF	Sold separately 80ng/ml bFGF should be supplemented	Sold separately 80ng/ml bFGF should be supplemented	Included
Use	Clinical research	Further manufacturing	Basic research & Clinical research
Number of Bottle	2	2	1

## » Differentiation supplement

#### Standard media for clinical research

**Animal Origin Free, Chemically defined** 

Efficient EB formation

Lineage-specific differentiation

#### Animal-Origin Free supplement

In the process of hPSC differentiation, BSA or other serum replacements containing animal derived ingredients have been traditionally used, which are known to carry a risk of hazardous viral contamination for cell therapy. Also, animal derived ingredients can cause the lot-to-lot variation which results in the manufacturing instability.

StemFit For Differentiation (StemFit For Diff.) is chemically defined and animal origin-free supplement for hPSC differentiation. StemFit For Diff is designed and manufactured under a strict animal origin free policy and is free from animal or human derived components.

StemFit For Diff. is desirable for CGT product manufacturing since its risk of viral contamination and lot-to-lot variation is minimized.



### • Lineage-specific differentiation under chemically-defined and AOF condition

StemFit For Diff. allows efficient directed differentiation for specific lineages (endoderm, mesoderm and ectoderm) under chemically defined and animal-origin free condition, which enables stable and clinically applicable differentiation for CGT products.



Further information is available on the webpage

Web site 🔶 🕨



StemFit For Differentiation

Serum-Free

Xeno-Free

Animal-Origin

Free

## » Mesenchymal Stem cell media

#### **Clinically applicable MSC media**

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PMDA consultation

**Excellent cell growth** 

**MSC** isolation from tissues



### Chemically defined & Regulatory compliance for CGT

Undefined extracts, such as FBS or human plate lysate, are known to carry a risk of hazardous viral contamination or lot-to-lot variation, which are not desirable for cell therapy. Even commercially available xeno-free media, whose formulation is not usually disclosed, could still contain such undefined extracts from human or animal sources.

StemFit For MSC media is engineered to be chemically defined, in which no undefined extracts are included, and all protein components are replaced with bacteria-derived recombinant proteins. With this chemically defined formulation, the risk of vial infection and lot-to-lot variation is minimized. Additionally, the Japanese PMDA has been consulted on the formulation and manufacturing process and found that StemFit For MSC satisfies all requirement of ancillary materials for cell and gene therapy (CGT).

### Excellent cell growth and MSC isolation

StemFit For MSC shows excellent and stable cell growth performance compared to FBS or hPL containing media. Also, StemFit For MSC allows the isolation of MSCs from tissues under chemically defined condition. These features enable efficient and clinically applicable MSC isolation and expansion.

## High performance

StemFit For MSC enables superior cell expansion compared to serum-containing media. This medium can maintain MSCs with high level of marker expression and differentiation potential.

#### » Expansion



\*We define "Chemically Defined Medium" as

1. A medium in which all of the components and concentrations are known.

A medium which does not contain serum, lysates, or other ingredients with unknown composition



As cell therapy development continues, the demand for recombinant proteins, such as growth factors and cytokines, is rapidly increasing for industrial use. In particular, recombinant proteins that are used in the manufacture of cell therapy products are required to have higher quality standards than conventional research reagents. This includes consistency between lots, and compliance with various laws and regulations.

StemFit Purotein®, whose name comes from Pure & Protein, is a brand of recombinant proteins applicable for use from the laboratory to GMP compliant manufacturing of cell therapy products.

All StemFit Purotein® products are manufactured free from animal or human derived components, which ensures high safety and lot-to-lot consistency. Additionally, our highly efficient protein expression system and purification flow enables high purity and quality while maintaining affordable prices. Furthermore, its frozen liquid form is a perfect match for advanced manufacturing because it enables the omission of the time-consuming reconstitution process.





### Affordable price

We are committed to technological innovation, production at the optimal scale, and cost reduction, so that we can always supply products at an affordable price.



## **Animal origin free**

Minimize risk of virus contamination and lot-to-lot variation with animal-origin free formula.



## Regulatory compliance for cell therapy products

The PMDA has officially confirmed our product's eligibility for use in clinical cell therapy production (in Japan)



## Frozen form that is "ready-to-use"

Can eliminate the time-consuming process of reconstitution, while ensuring consistent results.

## » High lot-to-lot consistency

### • Excellent Protein Expression System with C. glutamicum

### ······ Corynebacterium glutamicum ······



<Features>

Secretory expression system

Low impurities

• Gram-positive, no endotoxin

Long history for commercial production

Highly consistent and low-cost expression system

*Corynebacterium glutamicum* is a gram-positive, non-sporulating soil bacterium which has successfully been used for the industrial production of amino acids for more than 50 years. Since *C. gulutamicum* secretes expressed target protein into culture media, highly purified proteins can be obtained with a simple purification process. Additionally, gram-positive bacteria contribute low endotoxin products.

### • Cutting Edge Refolding Technology, FMR

Refolding is an important process in the production of recombinant proteins since it has a significant impact on the product quality. In the process, denatured protein and a refolding buffer are mixed in a flask in a strictly controlled manner. StemFit Purotein® is manufactured with an advanced refolding technology, FMR (Flow Microreactor), in which protein and buffer are continuously flowed and mixed in a micro space. FMR allows us to precisely control and optimize the mixing reaction at a micro-second scale, which enables efficient and consistent refolding between lots and different manufacturing scales.

What is FMR? Flow microreactor (FMR) is a flow reactor in which specific phenomena occur in micro space.



### Difference from conventional refolding technology



Patent pending (WO2020095894)

## » Animal-Origin Free & Regulatory Compliance for CGT

Animal or human derived components, such as serum derived albumin, or animal cell derived recombinant proteins, are known to carry a risk of hazardous viral contamination for cell therapy. Therefore, StemFit Purotein<sub>®</sub> is designed and manufactured under a strict animal origin free policy and is free from animal or human derived components according to the requirement of Japanese PMDA for ancillary materials. Additionally, StemFit Purotein<sub>®</sub> offers GMP compliant products that are manufactured under GMP guidelines and are thus preferable for cell therapy product manufacturing.



## » High purity & High performance

The secreting expression system of *C. glutamicum* enables the omission of the refolding process. For other expression systems, a cutting-edge refolding technology, FMR, enables high purity and high-performance protein production. These highly purified proteins minimize undesirable effects from impurities and ensure consistent lot-to-lot results during manufacturing.



Activin A from various suppliers are tested.

## » Frozen form, Ready to use

Conventional lyophilized products require reconstitution and concentration measurements which are time-consuming and introduce a risk of bacterial contamination or unexpected inactivation. StemFit Purotein<sub>®</sub> products are provided in a frozen liquid form which is ready to use and could omit reconstitution steps. This feature simplifies the cell manufacturing process and accelerates cell therapy projects.

### • Simplified protocols without reconstitution



## » High compatibility with StemFit hPSC culture media

Human pluripotent stem cells (hPSC) are promising resources for research and cell therapy since they can differentiate into various type of cells. In the directed differentiation process of hPSCs, cells undergo several differentiation steps towards the target tissues. During this process, recombinant proteins are added into the culture media as growth factors to stimulate differentiation. StemFit hPSC media is a brand of hPSC media suitable for all stages of research. It is also highly compatible with StemFit Purotein<sub>®</sub>. The combination of StemFit hPSC media and StemFit Purotein<sub>®</sub> allows for the establishment of highly efficient differentiation systems in the laboratory while ensuring an easy transition to GMP-compliant production for future cell therapy manufacturing.



## » Human bFGF recombinant



### What is **bFGF**?

FGF basic (bFGF) is a member of the FGF family of cytokines and is involved in a wide range of biological processes including proliferation of many type of cells, neuron differentiation and maintenance of pluripotent state. bFGF is mainly used for stem cell cultivation in order to expand pluripotent stem cells or mesenchymal stem cells. Human bFGF recombinant is a 17.1 kDa, containing 154 amino acid residues.



### » Product Details

#### NAME

Human bFGF recombinant

#### SOURCE

Corynebacterium glutamicum

#### FORMULATION

Frozen in PBS buffer containing 1 mM DTT

#### Quality

Animal origin free \*Produced in compliance with GMP.

#### AA Sequence:

AAGSITTLPA LPEDGGSGAF PPGHFKDPKR LYCKNGGFFL RIHPDGRVDG VREKSDPHIK LQLQAEERGV VSIKGVCANR YLAMKEDGRL LASKCVTDEC FFFERLESNN YNTYRSRKYT SWYVALKRTG QYKLGSKTGP GQKAILFLPM SAKS

Purity:

 $\geq$  95% by SDS-PAGE

Endotoxin <1EU/ug

Further information is available on the webpage

Web site 🕨 🕨

## » Recombinant human Activin A



### » Product Details

#### NAME

Recombinant human Activin A

#### SOURCE

E. coli

#### FORMULATION

Frozen in 50 mM sodium acetate buffer, pH4.6

#### Quality

Animal origin free

#### AA Sequence:

GLECDGKVNI CCKKQFFVSF KDIGWNDWII APSGYHANYC EGECPSHIAG TSGSSLSFHS TVINHYRMRG HSPFANLKSC CVPTKLRPMS MLYYDDGQNI IKKDIQNMIV EECGCS

#### Purity:

 $\geq$  97% by SDS-PAGE

### Endotoxin

<0.01EU/ug

### What is Activin A?

Activin A is a member of the TGF-beta superfamily of cytokines and is involved in a wide range of biological processes including tissue morphogenesis and repair, fibrosis, inflammation, neural development, hematopoiesis, reproductive system function, and carcinogenesis. Human Activin A is a 26.0 kDa disulfide-linked homodimer of two A chains, each containing 116 amino acid residues. Activin A is mainly used for stem cell cultivation in order to differentiate the stem cell into endoderm or mesoderm.

### » Comparison of MS spectrum with other companies' GMP products

AJINOMOTO





Company Y

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### » Comparison of biological activity with other companies' GMP products

Measured by its ability to induce hemoglobin expression in K562 human chronic myelogenous leukemia cell. Activin A WHO international std., NIBSC code: 91/626



AJINOMOTO's Activin A demonstrates equal to superior biological activity compared to tested alternatives!

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Further information is available on the webpage (Website

## » Recombinant Human SCF



### What is SCF?

SCF (stem cell factor) is a growth factor secreted from fibroblasts and endothelial cells. SCF binds to a receptor known as c-Kit and is thought to play an important role in the survival, proliferation and differentiation of hematopoietic stem cells. Recombinant Human SCF is an 18.5 kDa polypeptide containing 164 amino acid residues, which corresponds to the sequence of the secreted soluble form of SCF.



### » Product Details

#### NAME

Recombinant Human SCF

#### SOURCE

Corynebacterium glutamicum

#### FORMULATION

Frozen in 20 mM HEPES

#### Quality

Animal origin free

#### AA Sequence:

EGICRNRVTN NVKDVTKLVA NLPKDYMITL KYVPGMDVLP SHCWISEMVV QLSDSLTDLL DKFSNISEGL SNYSIIDKLV NIVDDLVECV KENSSKDLKK SFKSPEPRLF TPEEFFRIFN RSIDAFKDFV VASETSDCVV SSTLSPEKDS RVSVTKPFML PPVA

#### Purity:

 $\geq 95\%$  by SDS-PAGE

#### Endotoxin

<0.1EU/ug

# » Comparison of biological activity with other companies' products

Phosphorylation assay of c-KIT



### AJINOMOTO's SCF demonstrates equal to biological activity compared to tested alternatives!

Further information is available on the webpage

Web site 🔶 🕨

## » Recombinant Human KGF (FGF-7)



### What is KGF?

Keratinocyte Growth Factor (KGF) is one of 23 known members of the FGF family, and it is known as FGF-7. Proteins of this family play a central role during prenatal development, postnatal growth, and the regeneration of a variety of tissues, by promoting cellular proliferation and differentiation. KGF is a mitogen factor specific for epithelial cells and keratinocytes. Recombinant Human KGF is an 18.9 kDa protein consisting of 163 amino acid residues.



### » Product Details

#### NAME

Recombinant Human KGF (FGF-7)

#### SOURCE

Corynebacterium glutamicum

#### FORMULATION

Frozen in DPBS(-) containing 1.5mM DTT and 100mM Na<sub>2</sub>SO<sub>4</sub>

#### Quality

Animal origin free

#### AA Sequence:

CNDMTPEQMA TNVNCSSPER HTRSYDYMEG GDIRVRRLFC RTQWYLRIDK RGKVKGTQEM KNNYNIMEIR TVAVGIVAIK GVESEFYLAM NKEGKLYAKK ECNEDCNFKE LILENHYNTY ASAKWTHNGG EMFVALNQKG IPVRGKKTKK EQKTAHFLPM AIT

#### Purity:

 $\geq 95\%$  by SDS-PAGE

### Endotoxin

<0.1EU/ug

### » Comparison of biological activity with other companies' products



### AJINOMOTO's KGF demonstrates equal biological activity compared to tested alternatives!

Further information is available on the webpage

Web site 🔶 🕨

## » Recombinant Human VEGF 165



### What is VEGF?

VEGF (Vascular Endothelial Growth Factor) is a protein that plays an important role in angiogenesis, the formation of new blood vessels. It is secreted by cells as a signal to nearby cells to stimulate the growth of new blood vessels. VEGF also plays an important role in other physiological processes such as wound healing, embryonic development, and regulating the permeability of blood vessels. In addition, VEGF has been shown to be involved in the progression of many diseases, such as cancer, diabetic retinopathy, and cardiovascular diseases. Recombinant Human VEGF165 is a 38.2 kDa, disulfide-linked homodimeric protein consisting of two 165 amino acid polypeptide chains.

#### » Product Details

#### NAME

Recombinant Human VEGF 165

#### SOURCE

Corynebacterium glutamicum

#### FORMULATION

Frozen in 20 mM citric acid containing 20 mM GSSG

#### Quality

Animal origin free

#### AA Sequence:

APMAEGGGQN HHEVVKFMDV YQRSYCHPIE TLVDIFQEYP DEIEYIFKPS CVPLMRCGGC CNDEGLECVP TEESNITMQI MRIKPHQGQH IGEMSFLQHN KCECRPKKDR ARQENPCGPC SERRKHLFVQ DPQTCKCSCK NTDSRCKARQ LELNERTCRC DKPRR

#### Purity:

 $\geq$  95% by SDS-PAGE

#### Endotoxin

<0.1EU/ug

### » Comparison of biological activity with other companies' products

VEGFR2/NFAT reporter assay



### AJINOMOTO's VEGF demonstrates equal biological activity compared to tested alternatives!

Further information is available on the webpage (

Web site 🔹 🕨

## » Product list

	Please request pricin	g! •
Product	Information	
StemFit Basic03	hPSC expansion medium for clinical research	Web site
StemFit Basic03 GMP	hPSC expansion medium for clinical research and further manufacturing	Web site
StemFit Basic04 Complete Type	hPSC expansion medium for basic research and clinical research one bottle composition	Web site
StemFit For Differentiation	Differentiation supplement for hPSC	Web site
StemFit For Mesenchymal Stem cell	hMSC expansion medium	Web site

# 🐌 StemFit Purotein.

Product	Information	
Activin A	10µg, 50µg, 1mg(0.1mg/ml)	Web site
SCF	10µg, 50µg, 1mg(0.1mg/ml)	Web site
bFGF	1mg(0.3mg/ml)	Web site >
KGF	10µg, 50µg, 1mg(0.1mg/ml)	Web site >
VEGF	10µg, 50µg, 1mg(0.1mg/ml)	Web site

## » Publications and references



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	Web Site		

**Please request pricing!** 

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