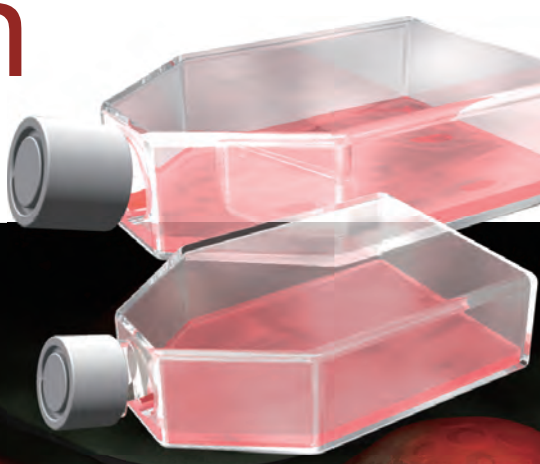
nacalai tesque
The quality for certainty.

Cell Cultivation

ver. 2.2



Contents

Introduction

Cell cultivation

Cell cultivation is to proliferate and/or maintain cells isolated from a host organism in nutrient medium, which means animal cell culture generally. Cells cultured in-vitro are called cultivated cells, and the nutrient medium for the cultivated cells is called a cell culture medium. Using cell cultivation, biological phenomena can be studied with simple experimental systems.

Cultivated cell types

Cultivated cells are classified as either adherent cells, which attach to a culture vessel, and suspension cells, which float freely in cell culture media. They are also divided into primary cells (first cells isolated from host organism) and cell lines (cells cultivated from primary cells long-term for a stable supply).

Sterilization

Bacteria, yeast and mycoplasma can grow very rapidly in nutrient-rich cell culture media. However, because they rapidly consume the nutrients of such cultures, the growth of target cells can be inhibited and cell death sometimes results due to toxic substances produced by microbes. To minimize this, a number of sterilization methods are available, including the following:

Method	Procedure	Target Object
Autoclave Sterilization	A holding time of at least 15 minutes at 120°C is required.	Materials not susceptible to heat denaturing, such as glass, heat-resistant plastic materials and salt solutions.
Dry-Heat Sterilization	A heating time of at least 90-120 minutes at 160°C or 45 minutes at 180°C is required.	Glass pipets, dishes, plates and trays used in CO_2 incubators. Note: Glass pipets should be cotton-plugged prior to treatment.
Filter Sterilization	A sterilized 0.2 μm filter can remove bacteria and yeast, but not viruses or certain mycoplasma. Sterilized disposable filtration devices can also be used.	Media or other substances that cannot be sterilized via the two methods above.

In addition to those mentioned above, ethylene oxide gas sterilization and UV sterilization are useful sterilization methods. Furthermore, sterilized disposable pipets and flasks are commonly used.

Media

Commonly used cell culture media are named by their developers, and setting appropriate cell culture conditions for each cell line is time-consuming and labor intensive work. Furthermore, because the concentration of each substance in a medium influences every other substance, if the concentration of a particular substance must be changed to reflect the particularities of a particular cell line, the concentrations of all other substances in a medium will normally require reconfirmation to ensure they are optimal as well. The most commonly used media are described below:

Name	Details	Target Cells
MEM (with Earle's salts)	MEM was developed by H. Eagle for use with the mouse L and Hela cell lines. This medium contains amino acids, vitamins, glucose and inorganic salts. Since MEM contains a minimal amount of amino acids, supplemental essential or non-essential amino acids may be required for particular cell lines.	Numerous cell lines
DMEM	The formula for DMEM was first published by Dulbecco *et al.* in 1959 for use with embryonal cell lines. DMEM is based on MEM and includes increased amounts of amino acids and vitamins. Dulbecco was awarded a Nobel Prize in Physiology or Medicine in 1975.	Human, mouse, rat, hamster, monkey and chicken cells.
Ham's F-12	Ham's F-12 was released by Ham in 1965 to improve the colony formation of CHO cells.	CHO cells
RPMI1640	RPMI1640 was developed by Roswell Park Memorial Institute（RPMI）based on McCoy5A. With the release of each improved version, a new numerical code was as appended to the name (i.e., 1629, 1630 and 1634). The characteristics of this media are marked by small amounts of Ca and Mg, and large amounts of inositol.	Suspension cells like human lymphoid cell, hybridoma cells, etc.

Serum

Serum, notably fetal bovine serum (FBS), the most common additive used in cell cultures, is a supernatant of clotted blood. Serum is added to media because it has growth factors, cytokines, nutrition factors, etc. that are not contained in cell cultivation supplements. Due to lot-to-lot variations, each FBS lot has to be tested in advance for cell line compatibility.

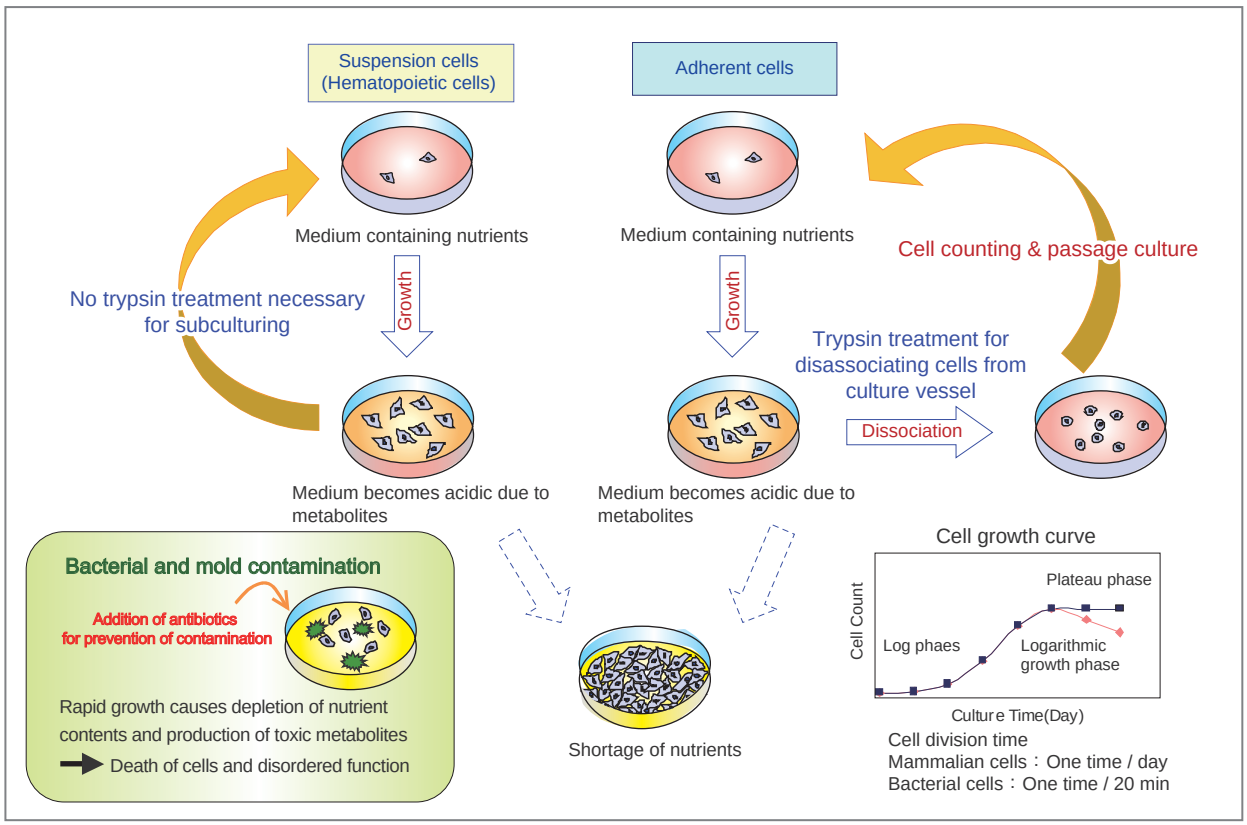
Serum free media

Media modified to cultivate cells without serum. Due to lack of serum, which consists of many kinds of factors, it is suitable for specific applications such as cellular response analysis and purification of proteins and bioactive components.

Cell Culture

Cells can maintain biological viability even after isolation from the host organism. Live cells flowing in blood vessels are cultured as suspension cultured cells, while those derived from tissues are cultured as adherent cultured cells. When a culture vessel becomes saturated with cells, nutrient shortages result and it becomes necessary to dissociate a number of cells from that vessel and reculture them in a new culture vessel with fresh media. This step is known as subculturing. The protocol below describes how to subculture adherent and suspension cultured cells.

How to culture cells



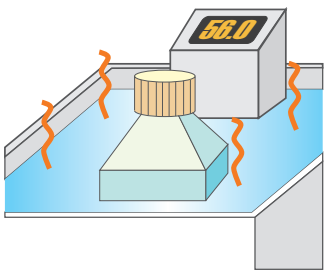
Protocol

1. Serum preparation (inactivation)

1-1. Melt frozen FBS by allowing the bottle to stand outside the freezer until it reaches room temperature.

1-2. Place the sealed bottle of melted FBS into a 56ºC water bath and allow it to heat until the FBS reaches a uniform temperature.

Note: Use care when placing the FBS bottle in the water bath to prevent it from breaking or leaking.

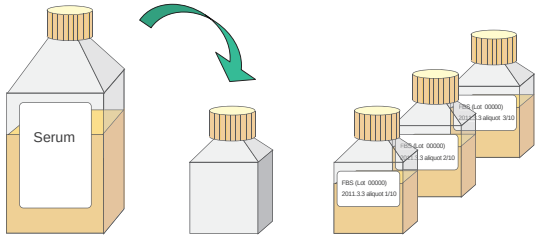


1-3. Incubate the FBS at 56ºC for 30 minutes.

Note:

FBS inactivation refers to the process by which undesired FBS elements that may cause cell damage are inactivated by heat treatment.

1-4. Dispense the heat-treated FBS into sterilized 55 ml bottles.



1-5. Store at -20ºC.

2. Preparation of medium including 10% FBS

Required Reagents

200mM-L-Glutamine Stock Solution (#16948-04)

Penicillin-Streptomycin Mixed Solution (Stabilized) (#09367-34)

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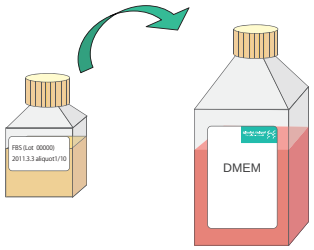
2-1. Add 55 ml of FBS to 500 ml of medium

Note: Make sure to measure and mix each batch precisely to ensure proper FBS concentrations.



2-2. If the medium selected for use does not include glutamine, add 200 mM of L-Glutamine solution.

Product Name	Product No.	Final concentration of L-Glutamine	Add 200 mM L-Glutamine solution to each 500 ml batch of medium
DMEM (4.5 g/l Glucose) without L-Gln and Sodium Pyruvate, liquid	08488-55	584 mg/l	10 ml
DMEM (4.5 g/l Glucose) without L-Gln, Sodium Pyruvate and Phenol red, liquid	08489-45		
DMEM (1.0 g/l Glucose) with Sodium Pyruvate, without L-Gln and Phenol Red, liquid	08490-05		

2-3. Add antibiotics to the medium as required

Product Name	Product No.	Recommended amount for 500 ml medium batch
Penicillin-Streptomycin Mixed Solution (Penicillin 5,000 u/ml, Streptomycin 5,000 µg/ml)	26252-94	5 ml
Penicillin-Streptomycin Mixed Solution (Penicillin 10,000 u/ml, Streptomycin 10,000 µg/ml)	26253-84	5 ml
Penicillin-Streptomycin Mixed Solution (Stabilized)	09367-34	5 ml
Antibiotic-Antimycotic Mixed Stock Solution (100x)	02892-54	5 ml
Gentamicin Sulfate Solution (10 mg/ml)	16672-04	2.5 ml

3-1. Subculturing adherent cells

Required Reagents

D-PBS(-)without Ca and Mg, liquid（#14249-95）

2.5g/l-Trypsin/1mmol/l-EDTA Solution, with Phenol Red（#32777-44）

DMEM（4.5g/l Glucose）with L-Gln, without Sodium Pyruvate, liquid（#08459-35）

RPMI 1640 with L-Gln, liquid（#30264-85）

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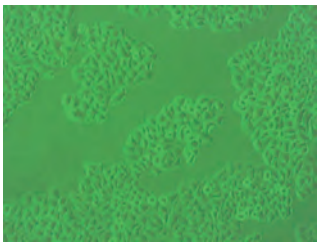
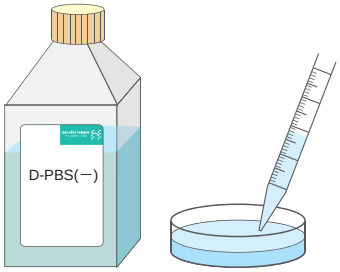
Since adherent cells are grown by attaching them to the culture dish and other cells, it is necessary to dissociate a number of cells via trypsin treatment when producing subcultures.

3-1-1. Confirm 70-80% cell confluence via microscopy.

**Confluence refers to the condition in which the entire culture dish surface is covered with cells.*

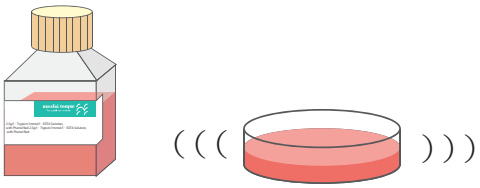


3-1-2. Carefully remove the medium from culture dish with an aspirator, then add D-PBS(-) gently from the side of the dish to wash the cells.

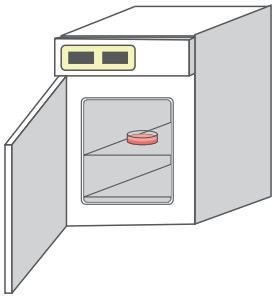


3-1-3. Add 3 ml of 2.5 g/l Trypsin-1 mmol/l EDTA solution and spread it evenly over the bottom of the entire dish.

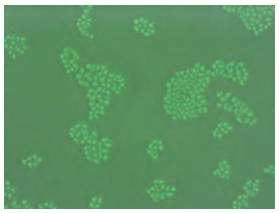
**Trypsin-EDTA solutions are useful for cell dissociation because trypsin decomposes proteins related to cell-cell and cell-dish adhesion, while EDTA facilitates and maintains trypsin activity by chelating the Mg and Ca ions.*



3-1-4. Place in incubator set at 37ºC.

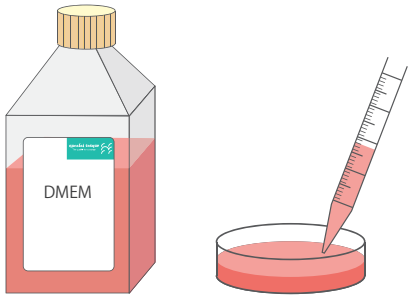


3-1-5. Check via microscopy to determine if cells are starting to curl, which indicates dissociation has begun.

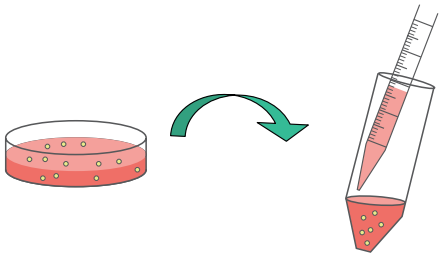


3-1-6. Add 10 ml of DMEM containing FBS and isolate a single cell from the agglomerated cells via pipetting.

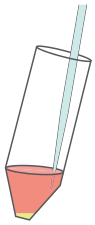
** Since FBS contains protease inhibitors, trypsin activity decreases.*



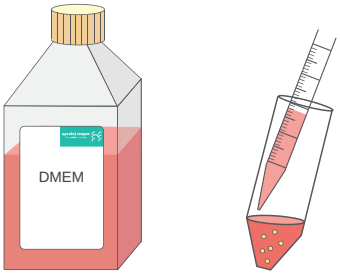
3-1-7. Transfer the suspension to a centrifuge tube.



3-1-8. Centrifuge at 1,000 rpm for 5 min. at 4ºC, and then remove the supernatant.



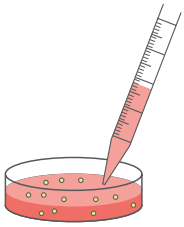
3-1-9. Add 5 ml of DMEM containing FBS to the centrifuge tube, then isolate a single cell from the agglomerated cells via pipetting.



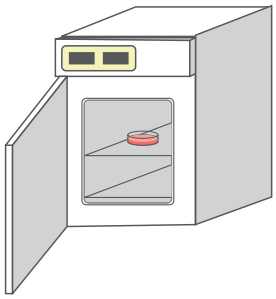
3-1-10. Count cells using the conventional method.

**Refer to page 12.*

3-1-11. Suspended cells should be precisely diluted to 5 x 10^4 cells/ml using DMEM containing FBS, after which 10 ml are seeded to each 100 mm dish.

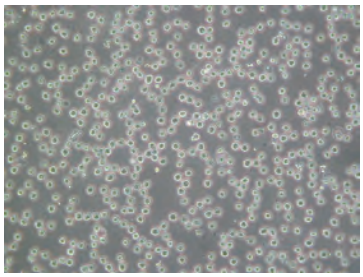


3-1-12. Incubate at 37ºC in a 5% CO_2 incubator.

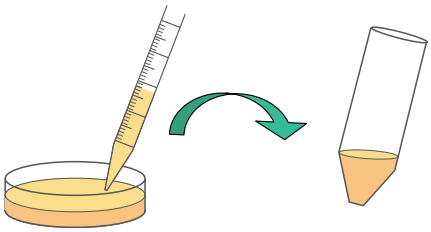


3-2. Subculturing suspension cells

3-2-1. Confirm 70-80% cell confluence via microscopy.



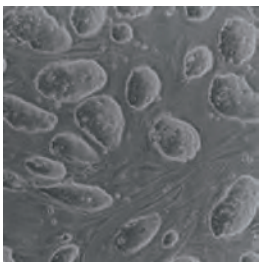
3-2-2. Gently perform pippetting to disperse cells in a cell culture dish, then transfer the suspension to a centrifuge tube.



3-2-3. Repeat the procedure from 3-1-8 described above.

Related Information

Feeder cells



Mouse ES cell culturing on feeder cells

In situations where cells are difficult to proliferate, feeder cells can be helpful for stimulating growth. The mouse embryonic fibroblast (MEF) and STO cell lines are commonly used to facilitate the growth and maintenance of ES/iPS cells in an undifferentiated state.

Mitomycin C Solution

Mitomycin C Solution
(#20898-21)

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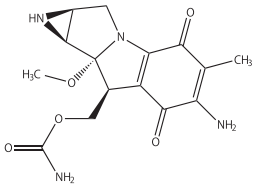

As mitomycin C makes cells stop proliferating by inhibition of DNA replication, it is useful for preparation of feeder cells, cell counting, etc. Mitomycin has low solubility in PBS and water, and precipitates when stored at -20°C; furthermore, the prepared solution is unstable (it keeps 90% of its activity for 3 days if stored at 4°C and 1 day at room temperature). Our mitomycin C solution, however, is stable for 2 years at -20°C with protection from light.

Leukemia Inhibitory Factor (LIF)

Recombinant Mouse LIF
(#07695-81 1.0 ml (106 units/ml))
(#07689-71 1.0 ml (107units/ml))

Recombinant Human LIF
(#07690-31 1.0 ml (106 units/ml))
(#07692-11 1.0 ml (0.5×107units/ml))

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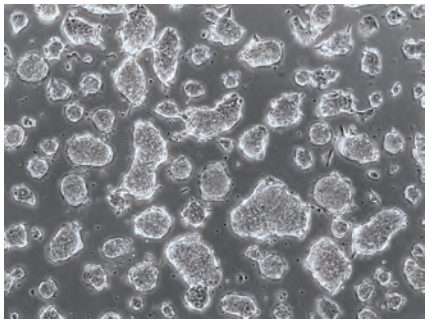
Leukemia Inhibitory Factor (LIF) is a well-known multi-function cytokine that has many uses including differentiation induction for neural stem cells, growth-stimulation for undifferentiated hematopoietic progenitor cells and differential inhibition for pluripotent stem cells. Application data for Nacalai Tesque's LIF used with mouse ES cells (CGR8) is shown below.

Application

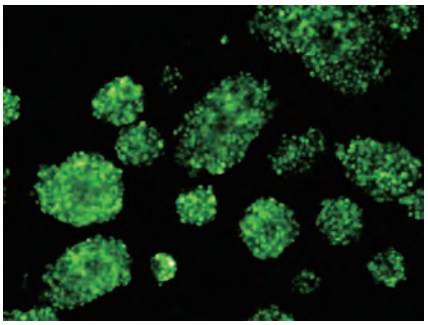
Culturing of mouse ES cells (CGR8)



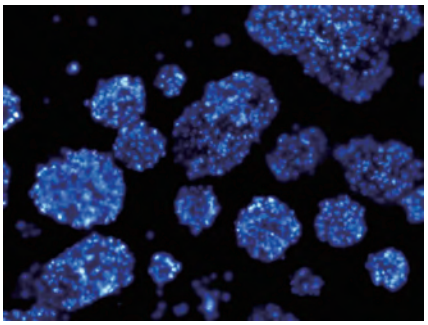
Detection of differentiation marker (Nanog)

Leukemia Inhibitory Factor (LIF) (Continued)

■ Nanog (Alexa Fluor®488)



■ Nucleus (DAPI)



Nanog proteins are detected on almost all cells.

Data courtesy of Teruhisa Kawamura, MD, PhD, Career-Path Promotion Unit for Young Life Scientists, Kyoto University

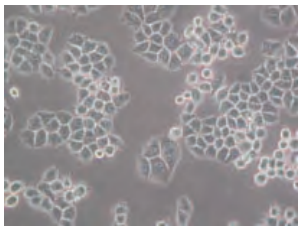
Contamination

Bacteria, fungi and yeasts

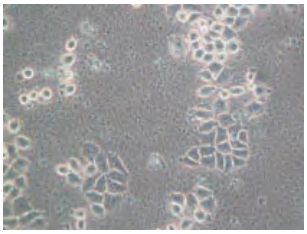
Determine if bacterial, fungal and/or yeast contamination is occurring by looking for the following signs:

- **pH value is lower than usual (medium color changes from red to yellow)**
- **Medium becomes cloudy**
- **Foreign substances are observed floating in medium**
- **Abnormal cell shapes observed under microscopy (refer to image below)**
- **Growth is extremely slow**

No contamination

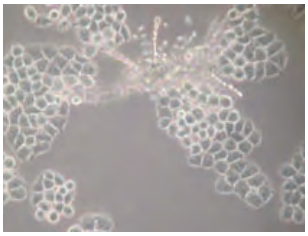


Bacterial contamination



Numerous small spots present

Fungal contamination



Threadlike lines observed

Immediately sterilize any contaminated media via autoclave and dispose of promptly.

Use antibiotics to prevent contamination by bacteria, fungi and yeasts

Antibiotics	M.W.	Action mechanisms	Targets	Solvent	Concentration
Amphotericin B	924.08	Cell membrane destruction	Fungus	DMSO DMF	2.5 µg/ml
Erythromycin	733.93	Inhibition of protein synthesis	Gram-positive Gram-negative Mycoplasma	HCl EtOH	100 µg/ml
Gentamicin	477.6	Inhibition of protein synthesis	Gram-positive Gram-negative	H_2O	50 µg/ml
Kanamycin Monosulfate	582.58	Inhibition of protein synthesis	Gram-positive Gram-negative Mycoplasma	H_2O	100 µg/ml
Penicillin G Potassium Salt	372.48	Inhibition of cell wall synthesis	Gram-positive	H_2O	100 U/ml
Streptomycin Sulfate	728.69	Inhibition of protein synthesis	Gram-positive Gram-negative	H_2O	100 µg/ml

Refer to page 33 for details on antibiotics

Cell Counting

The following cell counting kits are produced by Nacalai Tesque, Inc.

Product Name	0.5%-Trypan Blue Stain Solution	MTT Cell Count Kit	Cell Count Reagent SF
Product No.	29853-34, 100 ml Nacalai Online Catalog	23506-80, 1 kit (1000 tests) Nacalai Online Catalog	07553-15, 500 tests 07553-44, 2500 tests Nacalai Online Catalog
Product Appearance			
Features	• Facilitates counting of living cells. • Inexpensive, easy to use. • Unable to determine whether apoptosis is occurring in cells. • Some errors may be observed. • Unsuitable for high-throughput assays.	• Designed for live cell counting. • Possible to do high-throughput assays easily even with limited sample amounts.	• Facilitates counting of live cells with high sensitivity.

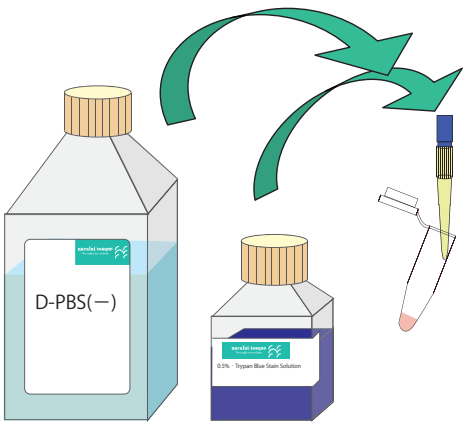
0.5%-Trypan Blue Stain Solution Product No. 29853-34

Protocol

1. Transfer 100 µl of cell suspension to a micro tube.
2. Determine cell density and add appropriate amount of 0.5%-Trypan Blue Stain Solution.

   E.g. Assay for 10x dilution:

Cell suspension	100 µl
D-PBS(-)	800 µl
0.5%-Trypan Blue Stain Solution	100 µl



3. Let stand for three minutes at room temperature after mixing by pipetting.

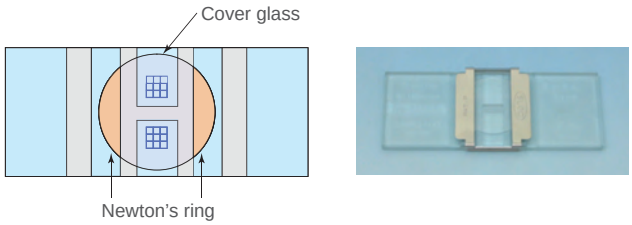
Note: Letting stand for long periods can stain even live cells.

4. Wash the counting chamber and cover glass with purified water and ethanol, then gently wipe away excess fluid with a Kimwipe®.

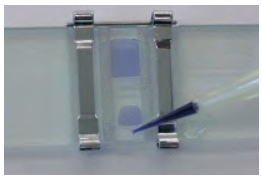
Note: Do not rub the lined areas vigorously because scuffs and abrasion could damage the gridlines.

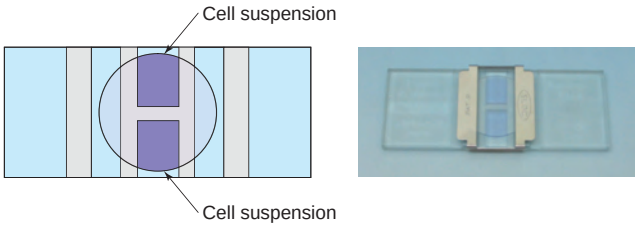
5. Set the cover glass on the counting chamber and slide it back and forth gently to eliminate air pockets. Then, press the cover glass against the counting chamber until Newton's rings appear (clamp with holdfast as desired).

Note: If Newton's rings do not appear, wash again or clean with alcohol wipes. Insufficient pressure bonding can cause leaks and result in incorrect counting.

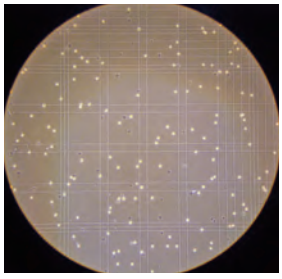


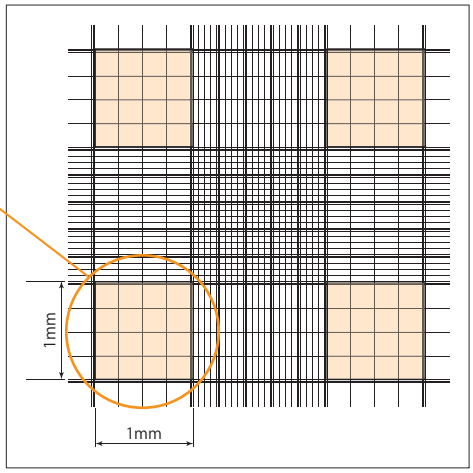
6. Inject the stained cell suspension between the counting chamber and the cover glass via capillary action using a micro pipette. To avoid incorrectly counting, make sure not to inject too much and form air bubbles.



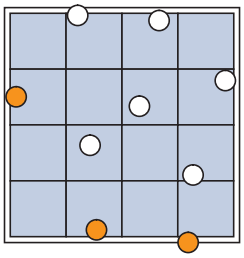


7. Count the number of cells present on each of the partitions in the four corners of the counting chamber (1 mm^2 partitions) with a microscope set at 100x magnification. For cells located on lines, determine which sides are to be counted in advance. For example, you may count cells present on the upper and right side lines, but not the bottom and left sides.


Microscope (×100)



Counting chamber



○ : Cells on upper and right side lines → Count
● : Cells on bottom and left side lines → Do not count

8. Calculate number of cell as follows.

Number of cells per ml = (total counted number of cells) / (counted number of partitions) x dilution ratio x 10^4
Total number of cells = number of cells per 1 ml x volume of cell suspension
Cell viability rate = number of living cells / total number of cells

For example
Volume of cell suspension: 5 ml
Dilution ratio: 10x
Counted number of partitions: 4

Result

Partition No.	1	2	3	4	Total
Number of living cells	37	40	42	41	160
Total number of cells	42	43	47	45	177

Number of cells = 160 / 4 × 10 × 10^4 = 4.0 × 10^6 cells/ml
Total number of cells = 4.0 × 10^6 cells/ml × 5 ml = 2.0 × 10^7 cells
Cell viability rate = 160 / 177 × 100 = 90%

FAQ

Question	Answer
Why does Trypan Blue stain dead cells and not living cells?	Trypan Blue is an azo colorant that combines strongly with proteins. Azo colorants suspended in a 0.01% saline solution penetrate dead cells via disrupted cell membranes; as living cells have intact cell membranes, they are not stained.
Can Trypan Blue be used to differentiate between living and dead plant cells?	Due to the strength of plant cell walls, staining dead plant cells is difficult.
Can Trypan Blue be used undiluted?	Yes, Trypan Blue can be used undiluted as its osmotic pressure is stable.

MTT Cell Count Kit Product No. 23506-80

Required Reagents

MTT Cell Count Kit
(#23506-80)

Nacalai Online Catalog

Protocols

Cell Proliferation Assay

1. Prepare the appropriate number of cells based on your experimental knowledge of the cell line, and then dispense 100 µl of the cell suspension into each well of a 96-well plate.
2. Incubate the plate for an appropriate amount of time in a CO_2 incubator.
3. Add 10 µl of MTT solution to each well.
4. Incubate for four hours.

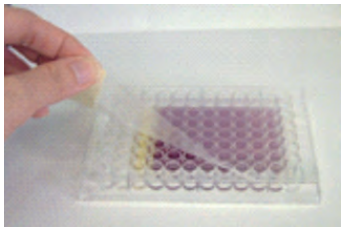
Note: Reaction times depend on the cell type and the number of cells, so preliminary testing is strongly recommended.

5. Add 100 µl of the Solubilization Solution (included in this kit) to each well.

Conventional method:

Dissolve precipitated formazans via pipetting.

Simplified method:



Seal the plate and incubate overnight at 37ºC.

Note:
Confirm that the plate seal is attached tightly to the plate. If the seal is incomplete, the Solubilization Solution will evaporate, and formazan precipitation will occur.
Although dissolution is possible when incubation is performed at room temperature, better results are achieved when incubating at 37ºC.
Depending on the type of plate seal, some evaporation might occur. If an incomplete seal is suspected, incubate the plate in a CO_2 incubator or other humid environment.

6. Measure absorbance at 570 nm (reference wavelength: 650 nm or higher) with a microplate reader.

Cell Cytotoxicity Assay

1. Prepare an appropriate number of cells based on your experimental knowledge of the cell line, and then dispense 100 µl of the cell suspension into each well of a 96-well plate.
2. Pre-incubate for 24 hours in a CO_2 incubator.
3. Add 10 µl of a dissolved target agent (for example, in PBS), to each well.
4. After incubating for an appropriate amount of time, add 10 µl of MTT solution to each well.
5. Incubate for four hours. Reaction times depend on the cell type and the number of cells, so preliminary testing is strongly recommended.
6. Add 100 µl of the Solubilization Solution (included in this kit) to each well.
   - Conventional method: Dissolve precipitated formazans via pipetting.
   - Simplified method: Seal the plate and incubate overnight at 37ºC.

Note:
Confirm that the plate seal is attached tightly to the plate. If the seal is incomplete, the Solubilization Solution will evaporate, and formazan precipitation will occur.
Although dissolution is possible when incubation is performed at room temperature, better results are achieved when incubating at 37ºC.
Depending on the type of plate seal, some evaporation might occur. If an incomplete seal is suspected, incubate the plate in a CO_2 incubator or other humid environment.

8. Measure absorbance at 570 nm (reference wavelength: 650 nm or higher) with a microplate reader.

Cell Count Reagent SF Product No, 07553-15 for 500 tests and 07553-44 for 2,500 tests

Required Reagents

Cell Count Reagent SF
(#07553-15 500TESTS)
(#07553-44 2500TESTS)

Nacalai Online Catalog

Protocols

Cell Proliferation Assay

1. Prepare an appropriate number of cells based on your experimental knowledge of the cell line, and then dispense 100 µl of the cell suspension into each well of a 96-well plate.

Note: Usable with media that contain phenol red

2. Pre-incubate in a CO_2 incubator.
3. Add 10 µl of Cell Count Reagent SF to each well of the cell culture plate.

Note: Sterilize the Cell Count Reagent with a 0.22 µm filter membrane if necessary.

4. Incubate for four hours in a CO_2 incubator.

Note:
Reaction times depend on the cell type and the number of cells, so preliminary testing is strongly recommended. After the color reaction is observed, stop the reaction process using one of the following procedures:

1) Cool the plate to 4°C.
2) Add 10 µl of 0.1 M HCl.
3) Add 10 µl of 1 w/v% SDS

Measure the absorbance within 24 hours.

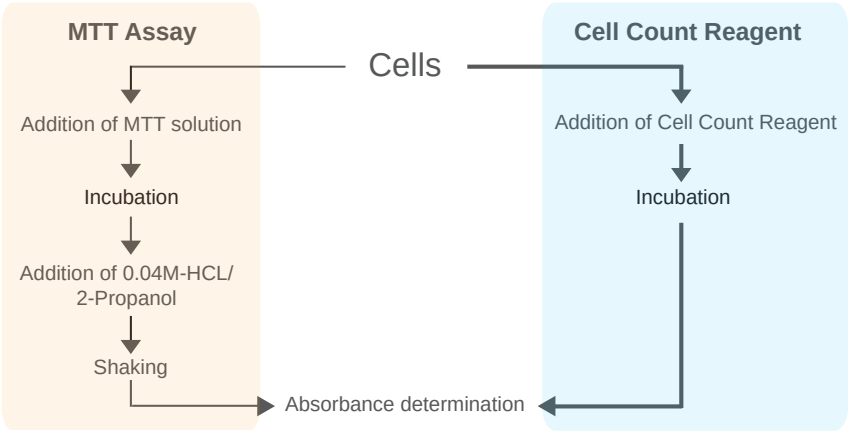
5. Measure absorbance at 450 nm (reference wavelength: 600 nm or higher) with a microplate reader.

Note: The maximum absorbance for formazan is around 460 nm, so use a 430-490 nm filter for high sensitivity.

Cell Cytotoxicity Assay

1. Prepare cells at a density of 5,000 cells per 100 µl, and then dispense 100 µl of the cell suspension into each well of a 96-well plate.
2. Pre-incubate for 24 hours in a CO_2 incubator.
3. Add 10 µl of a toxicant, prepared at the appropriate concentration, to each well.
4. Incubate for 48 hours in a CO_2 incubator.
5. Add 10 µl of Cell Count Reagent SF to each well.
6. Incubate from one to four hours in a CO_2 incubator.
7. Measure the absorbance at 450 nm (reference wavelength: 600 nm or higher) with a microplate reader.

Protocol comparison with MTT Assay and Cell Count Reagent SF



Cell Freezing Media

Freezing cells at -80ºC or using liquid nitrogen is helpful for preventing phenotypical changes and microbial contamination. Cell Reservoir One is a serum-free cell culture freezing medium containing Sericin that provides the same cryopreservation efficacy as FBS along with the reduced cell toxicity of DMSO. However, as DMSO is known to have adverse effects on certain cellular functions, two types of Cell Reservoir One, with and without DMSO, are offered.
Vitrification has become an important alternative to standard slow programmable freezing methods for cryopreservation of primate ES cell lines, including Human iPS cells, because it results in higher survival rates for cells after thawing. However, vitrification requires an ultra-rapid freezing protocol, usually less than 15 seconds between making cell suspensions and freezing in liquid nitrogen. Cell Reservoir One (Vitrify) is a novel serum-free cell culture freezing medium for use with the vitrification method. This culture contains Sericin, a water-soluble glycoprotein isolated from the silkworm cocoon, as a major constituent. Cell Reservoir One (Vitrify) provides high survival rates for primate cells, such as Monkey ES and Human iPS cells, even with a longer freezing protocol (up to 60 seconds from the cell collection to freezing in liquid nitrogen).

**Cell Reservoir One is produced in cooperation with Seiren Co. Ltd. (Patent pending)*

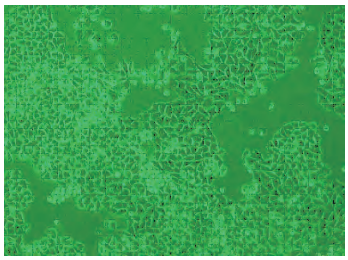
Product Name	Cell Reservoir One (with DMSO)	Cell Reservoir One (without DMSO)	Cell Reservoir One (Vitrify)
Method	Slow Freezing		Vitrification
Product No.	#07485-44 100 ml Nacalai Online Catalog	#07579-24 100 ml Nacalai Online Catalog	#11325-62 25 ml Nacalai Online Catalog
Product Appearance			
Features	• Applicable to ES cells (without DMSO) • High cell recovery and viability • Suitable for serum-free culture • Serum-free with no animal derived components		• Applicable to ES/iPS cells • High viability with a longer freezing protocol (up to 60 seconds) • Low toxicity to cells (DMSO and acetamide free)
Patent	Patented in Japan		Patent Pending

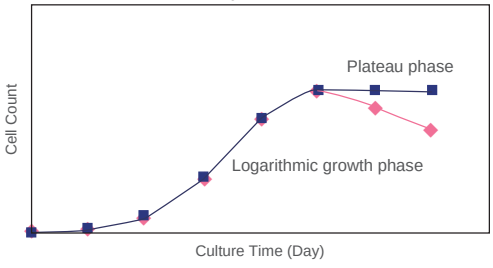
Cell Reservoir One (Slow Freezing Method) Product No. 07485-44

Protocol

1. Cell freezing with Cell Reservoir One (Slow-freezing method)

1-1. Verify that confluence is between 70 to 80% via microscopy, and then replace the medium.

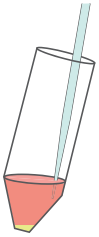




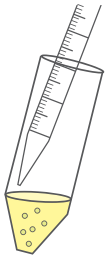
Note:
It is important to ensure that the cells are in the log phase of growth.

1-2. Pre-incubate for 24 hours in a CO_2 incubator.

1-3. Dissociate cells using the standard procedure.

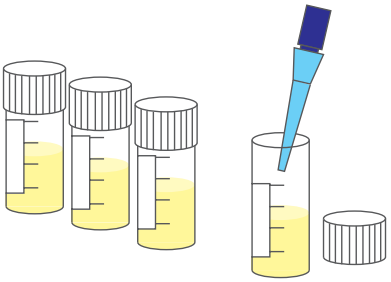


1-4. Centrifuge at 4ºC, 1000 rpm, for five min. Remove the supernatant with an aspirator.



1-5. Resuspend the cell pellet in Cell Reservoir One at 5 × 10^5 - 1 × 10^7 cells / ml cell density.

Note: To prevent cell damage, proceed through steps 5 to 7 as rapidly as possible.



1-6. Dispense the cell suspension into cryopreservation tubes.

1-7. Immediately store the prepared tubes at -80ºC.

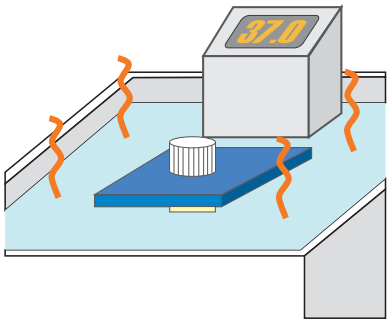
2. Cell Recovery

Required Reagents

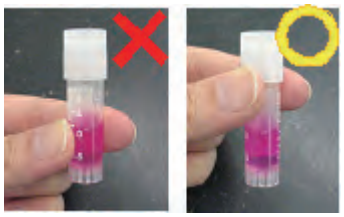
DMEM（4.5g/l Glucose）with L-Gln,
without Sodium Pyruvate, liquid（#08459-35）

Nacalai Online Catalog

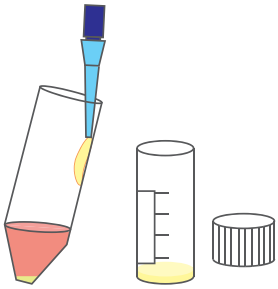
2-1. Remove the cryopreservation tubes from the freezer and immediately place in a 37ºC water bath.



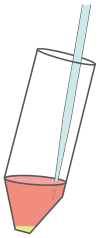
Note:
To prevent melting from body heat, avoid touching the side of the cryopreservation tube.



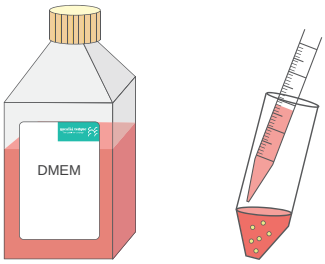
2-2. Resuspend the melted suspension in a centrifuge tube with 10 ml of DMEM containing FBS.



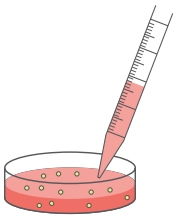
2-3. Centrifuge at 4ºC, 1000 rpm, for five min. Remove the supernatant with an aspirator.



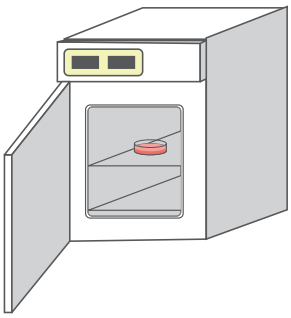
2-4. Resuspend the cell pellet in 10 ml of DMEM containing FBS.



2-5. Transfer to a 10 cm cell culture dish.



2-6. Incubate the dish in a 5% CO_2 incubator set at 37ºC.



FAQ

Question	Answer
Although Cell Reservoir One protocol calls for storage at 4ºC, is it possible to store at –20ºC?	We cannot guarantee its quality if stored at –20ºC.
Although Cell Reservoir One protocol calls for 1 ml of Cell Reservoir One for 5 × 10^5 - 1 × 10^7 cells, is it possible to reduce the amount of Cell Reservoir One for fewer cells? What is the minimum volume of Cell Reservoir One required?	As long as cell density is 5 × 10^5 - 1 × 10^7 cells/ml, reduction of volume of Cell Reservoir One should not be a problem.
Are there any data using Cell Reservoir One for HEK293 (MSR) cell line or for invertebrate cells?	No such data is currently available, but we have confirmed the efficacy of Cell Reservoir One with a number of human-derived cell lines such as Jurkat, Hela, HepG2 and others. For the HEK293 (MSR) cell line, cell viability was not found to decrease significantly compared to other human-derived cell lines. Regarding invertebrate cells, we have tested Cell Reservoir One using the Sf-9 cell line.
Does this conform to Good Laboratory Practices (GLP)?	No, it does not. This is for research use only.
Are there any data available on human-derived fibroblast cell and hematopoietic cell lines?	While data on human-derived fibroblast cell lines is not available, we have successfully tested the cell lines below: ・Mouse L929 ・Rabbit RC4 ・Mouse KUSA-A1 For human-derived hematopoietic cell lines, the following have been tested: ・WIL2-NS ・HL-60-RG ・Jurkat
Do you have comparison data on cells preserved for 12 months using conventional compositions (such as FBS containing 10% DMSO) and cells preserved with Cell Reservoir One?	Please see the following information on cell viability rates: For CHO DP-12: Cell Reservoir One: approx. 90% FBS with 10% DMSO: approx. 90% For HepG2 and Rin5F: Cell Reservoir One: approx. 80% FBS with 10% DMSO: approx. 80%
According to a particular reference, coating dishes with Sericin can increase cell growth. Is the high cell viability rate that results when freezing cells with Cell Reservoir One attributable to increased growth rates with Sericin?	While definite factors have yet to be clarified, the following effects have been attributed to Sericin: 1 Reduced stress during freezing and thawing due to Sericin's hydrophilicity. 2 Reduced DMSO toxicity. 3 Increased cell growth. A particular reference mentions that coating a dish with gelled high molecular weight Sericin can improve cell growth by increasing the adherence capability of the cells. However, this characteristic depends on the molecular weight of the Sericin used. The Sericin in Cell Reservoir One has a lower molecular weight than the one discussed in that reference.

Comparison data of Cell Reservoir One with DMSO and without DMSO

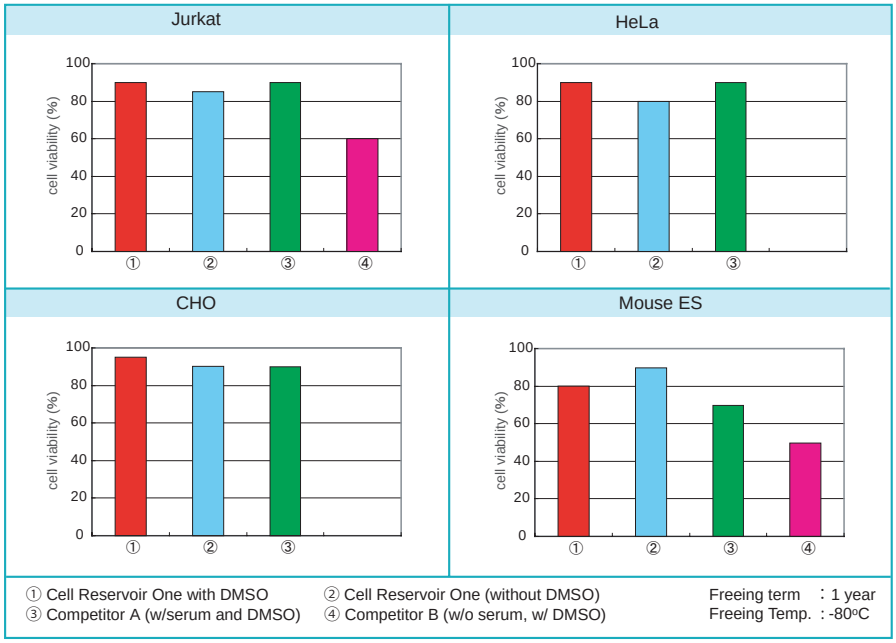
Required Reagents

Cell Reservoir One Trial Set (#09550-01)

Cell Reservoir One (#07579-24)

Cell Reservoir One (#07485-44)

As DMSO is known to have adverse effects on cellular functions during cell freezing, we offer two types of Cell Reservoir One: with and without DMSO.



Cell freezing with vitrification method Product No. 11325-62

Required Reagents

Cell Reservoir One, Vitrify (#11325-62))

Nacalai Online Catalog

Preparation

In order to facilitate high viability, it is important to freeze and thaw cells very rapidly. Accordingly, be sure to prepare all necessary materials and equipment before starting the freezing procedure.

Freezing protocol

1. Prepare tweezers and liquid nitrogen near a clean bench.
2. Detach the primate ES/iPS cells with a dissociation solution (0.25% trypsin/collagenase IV solution), and then carefully collect the solution, taking care not to break down the cell colonies.
3. Transfer the cell colonies into centrifuge tubes, and centrifuge them to remove as much supernatant as possible. *Diluting Cell Reservoir One (Vitrify) in the supernatant may decrease viability.
4. Add 200 µl of Cell Reservoir One (Vitrify), and carefully mix by pipetting four to five times, taking care not to disrupt cell colonies. Transfer the colonies quickly into a cryopreservation tube, and fasten the cap securely.

Note: Mix Cell Reservoir One (Vitrify) by pipetting before use.

5. Grasp the tube using tweezers and then insert two thirds of its length into the liquid nitrogen for 10 seconds, and then immerse it completely. For successful cryopreservation, steps (4)-(5) of this process should be performed within 60 seconds.
6. Transfer the tube into a liquid nitrogen storage tank.

Thawing protocol

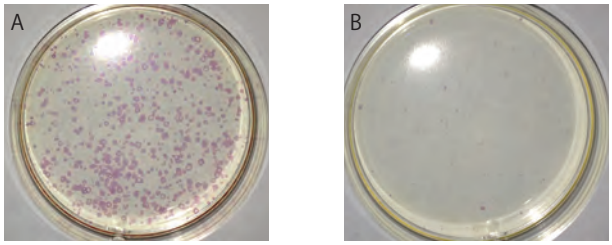
1. Pre-warm 10 ml of the appropriate cell culture medium in a centrifugation tube to 37°C.
2. Remove the cryopreservation tube containing the frozen cells from the liquid nitrogen storage tank and transfer it to a clean bench while leaving it immersed in liquid nitrogen.
3. Remove the tube from the liquid nitrogen. Open the cap and discard the liquid nitrogen in the tube by turning it upside down.
4. Thaw the cells quickly by adding more than 800 ml of the pre-warmed cell culture medium to the tube and pipetting a few times.
5. Transfer the cell suspension (4) to the centrifugation tube (1).
   *Perform operations (3)-(5) as rapidly as possible.
6. Wash the cryopreservation tube with cell culture medium, and transfer the medium to the centrifugation tube (5).
7. Remove as much supernatant as possible after centrifugation, and then seed cells into fresh medium.

Application

Comparison of survival rate of Human iPS cells (201B7 cell line*)

**Takahashi, K. et al. Cell, 2007 Nov 30; 131(5): 861-872*

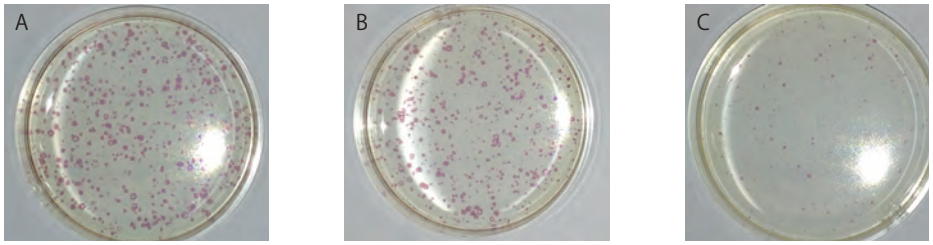
Freezing protocol: 60 seconds



A: Cell Reservoir One (Vitrify) B: DAP213

Human iPS cells were cryopreserved for more than two weeks in Cell Reservoir One (Vitrify) and DAP213. Viability was determined using alkaline phosphatase four days after thawing. Those cells preserved in Cell Reservoir One (Vitrify) showed high survival rates, while most of the cells preserved in DAP213 were dead.

Freezing protocol: 15 seconds



A: Cell Reservoir One (Vitrify) B: DAP213 C: Company A

Human iPS cells were cryopreserved for more than two weeks in Cell Reservoir One (Vitrify), DAP213 and Company A's product. Viability was determined using akaline phosphatase four days after thawing. Cell Reservoir One (Vitrify) showed the highest viability.

Data courtesy of a customer

	Freezing Medium	The Number of Colony		
		Vitrification Method		Slow Freezing method
		60 Seconds	15 Seconds	
A	Cell Reservoir One, Vitrify	672	563	-
B	DAP213	37	479	-
C	Company A	-	-	172

Appendix

List of Media (refer to page 25 for each composition)

Product Name	Grade	Storage	Product No.	PKG Size	Online Catalog
DMEM (No Glucose) with L-Gln, without Sodium Pyruvate, liquid	SP (for TC)	R	09891-25	500 ml	
DMEM (1.0g/l Glucose) with L-Gln and Sodium Pyruvate, liquid	SP (for TC)	R	08456-65	500 ml	
			08456-36	10 x 500 ml	
DMEM (1.0g/l Glucose) with Sodium Pyruvate, without L-Gln and Phenol Red, liquid	SP (for TC)	R	08490-05	500 ml	
DMEM (4.5g/l Glucose) with L-Gln and HEPES, without Sodium Pyruvate, liquid	SP (for TC)	R	08457-55	500 ml	
DMEM (4.5g/l Glucose) with L-Gln and Sodium Pyruvate, liquid	SP (for TC)	R	08458-45	500 ml	Nacalai Online Catalog
			08458-16	10 x 500 ml	
DMEM (4.5g/l Glucose) with L-Gln, without Sodium Pyruvate, liquid	SP (for TC)	R	08459-35	500 ml	
			08459-64	10 x 500 ml	
DMEM (4.5g/l Glucose) with HEPES, without L-Gln and Sodium Pyruvate, liquid	SP (for TC)	R	11585-75	500 ml	
DMEM (4.5g/l Glucose) with Sodium Pyruvate, without L-Gln, liquid	SP (for TC)	R	11584-85	500 ml	
DMEM (4.5g/l Glucose) without L-Gln, Sodium Pyruvate and Phenol Red, liquid	SP (for TC)	R	08489-45	500 ml	
DMEM (4.5g/l Glucose) without L-Gln and Sodium Pyruvate, liquid	SP (for TC)	R	08488-55	500 ml	
DMEM/Ham's F-12 with L-Gln, Sodium Pyruvate and HEPES, liquid	SP (for TC)	R	08460-95	500 ml	
DMEM/Ham's F-12 with L-Gln, Sodium Pyruvate and HEPES, without Phenol Red, liquid	SP (for TC)	R	05177-15	500 ml	
DMEM/Ham's F-12 with L-Gln and Sodium Pyruvate, without HEPES, liquid	SP (for TC)	R	11581-15	500 ml	
DMEM/Ham's F-12 with L-Gln and Sodium Pyruvate, without HEPES and Phenol Red, liquid	SP (for TC)	R	11582-05	500 ml	Nacalai Online Catalog
DMEM/Ham's F-12 with Sodium Pyruvate and HEPES, without L-Gln, liquid	SP (for TC)	R	11583-95	500 ml	
DMEM/Ham's F-12 (No Glucose) with L-Gln and Sodium Pyruvate, liquid	SP (for TC)	R	09893-05	500 ml	
G-MEM with L-Gln, liquid	SP (for TC)	R	12965-65	500 ml	
Ham's F-12 with L-Gln, liquid	SP (for TC)	R	17458-65	500 ml	
IMDM withL-Gln and HEPES, liquid (Iscove's Modified Dulbecco's Medium)	SP (for TC)	R	11506-05	500 ml	
MEM with Earle's Salts and L-Gln, liquid	SP (for TC)	R	21442-25	500 ml	
MEM with Earle's Salts, L-Gln and Non-Essential Amino Acids, liquid	SP (for TC)	R	21443-15	500 ml	
MEM (No Glucose) with Earle's Salts, L-Gln and Non-Essential Amino Acids, liquid	SP (for TC)	R	09848-05	500 ml	
α-MEM with L-Gln, Ribonucleosides and Deoxyribonucleosides, liquid	SP (for TC)	R	21444-05	500 ml	
α-MEM with L-Gln, without Ribonucleosides and Deoxyribonucleosides, liquid	SP (for TC)	R	21445-95	500 ml	
RPMI 1640 with L-Gln, liquid	SP (for TC)	R	30264-85	500 ml	
			30264-56	10x 500 ml	Nacalai Online Catalog
RPMI 1640 with L-Gln and HEPES, liquid	SP (for TC)	R	30263-95	500 ml	
RPMI 1640 with L-Gln, without Phenol Red, liquid	SP (for TC)	R	06261-65	500 ml	
RPMI 1640 without L-Gln, liquid	SP (for TC)	R	05176-25	500 ml	
RPMI 1640 (No Glucose) with L-Gln, liquid	SP (for TC)	R	09892-15	500 ml	

[Storage] R = Refrigerator

Custom Service

Nacalai Tesque offers custom service for cell culture media according to your personal requirements.

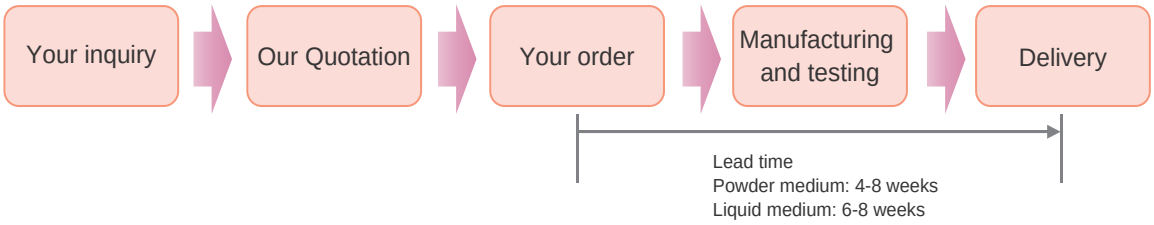
Have you ever wanted to…

- **try a medium of a particular composition mentioned in a journal?**
- **remove a particular amino acid?**
- **change the concentration of a vitamin?**
- **change the composition of a medium?**
- **remove the estrogenic activity caused by phenol red?**

Basic conditions

Product Form	Powder	Liquid
Minimum Quantity	500 g	500 ml
Packing Material	Glass Bottle	PET Bottle（100 ml / 500 ml）
Guarantee of Quality	・pH ・Osmotic Pressure ・Endotoxin Test	・pH ・Osmotic Pressure ・Sterilization Test ・Endotoxin Test ・Mycoplasma Test
Delivery Time	4-8 weeks	6-8 weeks

Order Flow



How to Order

Please vist our website at *http://www.nacalai.co.jp/global/reagent/custom/Custom_Services.html* and fill out request form.

Nacalai Custom

List of media components

DMEM

Nacalai Online Catalog

Product No.	09891	08456	08490	08457	08458
Media	DMEM (No Glucose)	DMEM (Low Glucose)	DMEM (Low Glucose)	DMEM (High Glucose)	DMEM (High Glucose)
Inorganic Salts:					
Calcium Chloride・2H_2O	265.00	265.00	265.00	265.00	265.00
Iron (III) Nitrate・9H_2O	0.10	0.10	0.10	0.10	0.10
Magnesium Sulfate	97.67	97.67	97.67	97.67	97.67
Potassium Chloride	400.00	400.00	400.00	400.00	400.00
Sodium Chloride	6400.00	6400.00	6400.00	4750.00	6400.00
Sodium Dihydrogenphosphate	109.00	109.00	109.00	109.00	109.00
Sodium Hydrogen Carbonate	3700.00	3700.00	3700.00	3700.00	3700.00
Amino Acids:					
L-Arginine・HCl	84.00	84.00	84.00	84.00	84.00
L-Cystine・2HCl	62.60	62.60	62.60	62.60	62.60
L-Glutamine	584.00	584.00	-	584.00	584.00
Glycine	30.00	30.00	30.00	30.00	30.00
L-Histidine・HCl・H_2O	42.00	42.00	42.00	42.00	42.00
L-Isoleucine	105.00	105.00	105.00	105.00	105.00
L-Leucine	105.00	105.00	105.00	105.00	105.00
L-Lysine・HCl	146.00	146.00	146.00	146.00	146.00
L-Methionine	30.00	30.00	30.00	30.00	30.00
L-Phenylalanine	66.00	66.00	66.00	66.00	66.00
L-Serine	42.00	42.00	42.00	42.00	42.00
L-Threonine	95.00	95.00	95.00	95.00	95.00
L-Tryptophan	16.00	16.00	16.00	16.00	16.00
L-Tyrosine・2Na・2H_2O	104.00	104.00	104.00	104.00	104.00
L-Valine	94.00	94.00	94.00	94.00	94.00
Vitamins:					
Choline Chloride	4.00	4.00	4.00	4.00	4.00
Folic Acid	4.00	4.00	4.00	4.00	4.00
myo-Inositol	7.20	7.20	7.20	7.20	7.20
Nicotinamide	4.00	4.00	4.00	4.00	4.00
D-Pantothenic Acid Calcium Salt	4.00	4.00	4.00	4.00	4.00
Pyridoxine・HCl	4.00	4.00	4.00	4.00	4.00
Vitamin B_1・HCl	4.00	4.00	4.00	4.00	4.00
Vitamin B_2	0.40	0.40	0.40	0.40	0.40
Others:					
D-Glucose	-	1000.00	1000.00	4500.00	4500.00
HEPES	-	-	-	5958.00	-
Phenol Red	14.93	14.93	-	14.93	14.93
Sodium Pyruvate	-	110.00	110.00	-	110.00

Concentration shown in mg/l

List of media components (Continued)

DMEM

Nacalai Online Catalog

Product No.	08459	11585	11584	08489	08488
Media	DMEM (High Glucose)	DMEM (High Glucose)	DMEM (High Glucose)	DMEM (High Glucose)	DMEM (High Glucose)
Inorganic Salts:					
Calcium Chloride • $2H_2O$	265.00	265.00	265.00	265.00	265.00
Iron (III) Nitrate • $9H_2O$	0.10	0.10	0.10	0.10	0.10
Magnesium Sulfate	97.67	97.67	97.67	97.67	97.67
Potassium Chloride	400.00	400.00	400.00	400.00	400.00
Sodium Chloride	6400.00	4750.00	6400.00	6400.00	6400.00
Sodium Dihydrogenphosphate	109.00	109.00	109.00	109.00	109.00
Sodium Hydrogen Carbonate	3700.00	3700.00	3700.00	3700.00	3700.00
Amino Acids:					
L-Arginine • HCl	84.00	84.00	84.00	84.00	84.00
L-Cystine • 2HCl	62.60	62.60	62.60	62.60	62.60
L-Glutamine	584.00	-	-	-	-
Glycine	30.00	30.00	30.00	30.00	30.00
L-Histidine • HCl • H_2O	42.00	42.00	42.00	42.00	42.00
L-Isoleucine	105.00	105.00	105.00	105.00	105.00
L-Leucine	105.00	105.00	105.00	105.00	105.00
L-Lysine • HCl	146.00	146.00	146.00	146.00	146.00
L-Methionine	30.00	30.00	30.00	30.00	30.00
L-Phenylalanine	66.00	66.00	66.00	66.00	66.00
L-Serine	42.00	42.00	42.00	42.00	42.00
L-Threonine	95.00	95.00	95.00	95.00	95.00
L-Tryptophan	16.00	16.00	16.00	16.00	16.00
L-Tyrosine • 2Na • $2H_2O$	104.00	104.00	104.00	104.00	104.00
L-Valine	94.00	94.00	94.00	94.00	94.00
Vitamins:					
Choline Chloride	4.00	4.00	4.00	4.00	4.00
Folic Acid	4.00	4.00	4.00	4.00	4.00
myo-Inositol	7.20	7.20	7.20	7.20	7.20
Nicotinamide	4.00	4.00	4.00	4.00	4.00
D-Pantothenic Acid Calcium Salt	4.00	4.00	4.00	4.00	4.00
Pyridoxine • HCl	4.00	4.00	4.00	4.00	4.00
Vitamin B_1 • HCl	4.00	4.00	4.00	4.00	4.00
Vitamin B_2	0.40	0.40	0.40	0.40	0.40
Others:					
D-Glucose	4500.00	4500.00	4500.00	4500.00	4500.00
HEPES	-	5958.00	-	-	-
Phenol Red	14.93	14.93	14.93	-	14.93
Sodium Pyruvate	-	-	110.00	-	-

Concentration shown in mg/l

DMEM/Ham's F-12

Nacalai Online Catalog

Product No.	08460	05177	11581	11582
Media	DMEM/Ham's F-12	DMEM/Ham's F-12	DMEM/Ham's F-12	DMEM/Ham's F-12
Inorganic Salts:				
Calcium Chloride・2H_2O	154.52	154.52	154.52	154.52
Copper (II) Sulfate・5H_2O	0.0013	0.0013	0.0013	0.0013
Iron (III) Nitrate・9H_2O	0.05	0.05	0.05	0.05
Iron (II) Sulfate・7H_2O	0.42	0.42	0.42	0.42
Magnesium Chloride・6H_2O	61.20	61.20	61.20	61.20
Magnesium Sulfate	48.84	48.84	48.84	48.84
Potassium Chloride	311.80	311.80	311.80	311.80
Sodium Chloride	6996.00	6996.00	6996.00	6996.00
Sodium Dihydrogenphosphate	54.30	54.30	54.30	54.30
Sodium Hydrogen Carbonate	1200.00	1200.00	2,438.00	2,438.00
di-Sodium Hydrogenphosphate	71.02	71.02	71.02	71.02
Zinc Sulfate・7H_2O	0.43	0.43	0.43	0.43
Amino Acids:				
L-Alanine	4.45	4.45	4.45	4.45
L-Arginine・HCl	147.50	147.50	147.50	147.50
L-Asparagine・H_2O	7.50	7.50	7.50	7.50
L-Aspartic Acid	6.65	6.65	6.65	6.65
L-Cysteine・HCl・H_2O	17.56	17.56	17.56	17.56
L-Cystine・2HCl	31.29	31.29	31.29	31.29
L-Glutamic Acid	7.35	7.35	7.35	7.35
L-Glutamine	365.00	365.00	365.00	365.00
Glycine	18.75	18.75	18.75	18.75
L-Histidine・HCl・H_2O	31.48	31.48	31.48	31.48
L-Isoleucine	54.47	54.47	54.47	54.47
L-Leucine	59.05	59.05	59.05	59.05
L-Lysine・HCl	91.25	91.25	91.25	91.25
L-Methionine	17.24	17.24	17.24	17.24
L-Phenylalanine	35.48	35.48	35.48	35.48
L-Proline	17.25	17.25	17.25	17.25
L-Serine	26.25	26.25	26.25	26.25
L-Threonine	53.45	53.45	53.45	53.45
L-Tryptophan	9.02	9.02	9.02	9.02
L-Tyrosine・2Na・2H_2O	55.79	55.79	55.79	55.79
L-Valine	52.85	52.85	52.85	52.85
Vitamins:				
D-Biotin	0.0035	0.0035	0.0035	0.0035
Choline Chloride	8.98	8.98	8.98	8.98
Folic Acid	2.66	2.66	2.66	2.66
myo-Inositol	12.60	12.60	12.60	12.60
Nicotinamide	2.02	2.02	2.02	2.02
D-Pantothenic Acid Calcium Salt	2.24	2.24	2.24	2.24
Pyridoxine・HCl	2.03	2.03	2.03	2.03
Vitamin B_1・HCl	2.17	2.17	2.17	2.17
Vitamin B_2	0.22	0.22	0.22	0.22
Vitamin B_{12}	0.68	0.68	0.68	0.68
Others:				
D-Glucose	3151.00	3151.00	3151.00	3151.00
HEPES	3574.50	3574.50	-	-
Hypoxanthine	2.10	2.10	2.10	2.10
Linoleic Acid	0.042	0.042	0.042	0.042
Lipoic Acid	0.11	0.11	0.11	0.11
Phenol Red	8.10	-	8.10	-
Putrescine・2HCl	0.081	0.081	0.081	0.081
Sodium Pyruvate	55.00	55.00	55.00	55.00
Thymidine	0.37	0.37	0.37	0.37

Concentration shown in mg/l

List of media components (Continued)

Nacalai Online Catalog

● Liquid Form of DMEM/Ham's F-12

● Ham's F-12

Product No.	11583	09893	17458
Media	DMEM/Ham's F-12	DMEM/Ham's F-12 (No Glucose)	Ham's F-12
Inorganic Salts:			
Calcium Chloride・2H_2O	154.52	154.52	44.10
Copper (II) Sulfate・5H_2O	0.0013	0.0013	0.0025
Iron (III) Nitrate・9H_2O	0.05	0.05	-
Iron (II) Sulfate・7H_2O	0.42	0.42	0.834
Magnesium Chloride・6H_2O	61.20	61.20	123.00
Magnesium Sulfate	48.84	48.84	-
Potassium Chloride	311.80	311.80	224.00
Sodium Chloride	6996.00	6996.00	7599.00
Sodium Dihydrogenphosphate	54.30	54.30	-
Sodium Hydrogen Carbonate	1200.00	1200.00	1176.00
di-Sodium Hydrogenphosphate	71.02	71.02	142.04
Zinc Sulfate・7H_2O	0.43	0.43	0.863
Amino Acids:			
L-Alanine	4.45	4.45	9.00
L-Arginine・HCl	147.50	147.50	211.00
L-Asparagine・H_2O	7.50	7.50	15.01
L-Aspartic Acid	6.65	6.65	13.30
L-Cysteine・HCl・H_2O	17.56	17.56	35.00
L-Cystine・2HCl	31.29	31.29	-
L-Glutamic Acid	7.35	7.35	14.70
L-Glutamine	-	365.00	146.00
Glycine	18.75	18.75	7.51
L-Histidine・HCl・H_2O	31.48	31.48	20.96
L-Isoleucine	54.47	54.47	3.94
L-Leucine	59.05	59.05	13.10
L-Lysine・HCl	91.25	91.25	36.50
L-Methionine	17.24	17.24	4.48
L-Phenylalanine	35.48	35.48	4.96
L-Proline	17.25	17.25	34.50
L-Serine	26.25	26.25	10.50
L-Threonine	53.45	53.45	11.90
L-Tryptophan	9.02	9.02	2.04
L-Tyrosine・2Na・2H_2O	55.79	55.79	7.78
L-Valine	52.85	52.85	11.70
Vitamins:			
D-Biotin	0.0035	0.0035	0.0073
Choline Chloride	8.98	8.98	13.96
Folic Acid	2.66	2.66	1.32
myo-Inositol	12.60	12.60	18.00
Nicotinamide	2.02	2.02	0.037
D-Pantothenic Acid Calcium Salt	2.24	2.24	0.48
Pyridoxine・HCl	2.03	2.03	0.062
Vitamin B_1・HCl	2.17	2.17	0.34
Vitamin B_2	0.22	0.22	0.038
Vitamin B_{12}	0.68	0.68	1.36
Others:			
D-Glucose	3151.00	-	1802.00
HEPES	3574.50	-	-
Hypoxanthine	2.10	2.10	4.08
Linoleic Acid	0.042	0.042	0.084
Lipoic Acid	0.11	0.11	0.21
Phenol Red	8.10	8.10	1.30
Putrescine・2HCl	0.081	0.081	0.161
Sodium Pyruvate	55.00	55.00	110.00
Thymidine	0.37	0.37	0.73

*Concentration shown in mg/l

Nacalai Online Catalog

● MEM

● α-MEM

Product No.	21442	21443	09848	21444	21445
Media	MEM	MEM	MEM (No Glucose)	α- MEM	α- MEM
Inorganic Salts:					
Calcium Chloride・2H$_2$O	265.00	265.00	265.00	265.00	265.00
Magnesium Sulfate	97.67	97.67	97.67	97.67	97.67
Potassium Chloride	400.00	400.00	400.00	400.00	400.00
Sodium Chloride	6800.00	6800.00	6800.00	6800.00	6800.00
Sodium Dihydrogenphosphate	122.00	122.00	122.00	122.00	122.00
Sodium Hydrogen Carbonate	2200.00	2200.00	2200.00	2200.00	2200.00
Amino Acids:					
L-Alanine	-	8.90	8.90	25.00	25.00
L-Arginine・HCl	126.00	126.00	126.00	126.00	126.00
L-Asparagine・H$_2$O	-	15.00	15.00	50.00	50.00
L-Aspartic Acid	-	13.30	13.30	30.00	30.00
L-Cysteine・HCl・H$_2$O	-	-	-	100.00	100.00
L-Cystine・2HCl	31.30	31.30	31.30	31.30	31.30
L-Glutamic Acid	-	14.70	14.70	75.00	75.00
L-Glutamine	292.00	292.00	292.00	292.00	292.00
Glycine	-	7.50	7.50	50.00	50.00
L-Histidine・HCl・H$_2$O	42.00	42.00	42.00	42.00	42.00
L-Isoleucine	52.00	52.00	52.00	52.00	52.00
L-Leucine	52.00	52.00	52.00	52.00	52.00
L-Lysine・HCl	72.50	72.50	72.50	72.50	72.50
L-Methionine	15.00	15.00	15.00	15.00	15.00
L-Phenylalanine	32.00	32.00	32.00	32.00	32.00
L-Proline	-	11.50	11.50	40.00	40.00
L-Serine	-	10.50	10.50	25.00	25.00
L-Threonine	48.00	48.00	48.00	48.00	48.00
L-Tryptophan	10.00	10.00	10.00	10.00	10.00
L-Tyrosine・2Na・2H$_2$O	51.90	51.90	51.90	51.90	51.90
L-Valine	46.00	46.00	46.00	46.00	46.00
Vitamins:					
L-Ascorbic Acid	-	-	-	50.00	50.00
D-Biotin	-	-	-	0.10	0.10
Choline Chloride	1.00	1.00	1.00	1.00	1.00
Folic Acid	1.00	1.00	1.00	1.00	1.00
myo-Inositol	2.00	2.00	2.00	2.00	2.00
Nicotinamide	1.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid Calcium Salt	1.00	1.00	1.00	1.00	1.00
Pyridoxal・HCl	1.00	1.00	1.00	1.00	1.00
Vitamin B$_1$・HCl	1.00	1.00	1.00	1.00	1.00
Vitamin B$_2$	0.10	0.10	0.10	0.10	0.10
Vitamin B$_{12}$	-	-	-	1.36	1.36
Others:					
Adenosine	-	-	-	10.00	-
Cytidine	-	-	-	10.00	-
2'-Deoxyadenosine・H$_2$O	-	-	-	10.72	-
2'-Deoxycytidine・HCl	-	-	-	11.00	-
2'-Deoxyguanosine	-	-	-	10.00	-
D-Glucose	1000.00	1000.00	-	1000.00	1000.00
Guanosine	-	-	-	10.00	-
Lipoic Acid	-	-	-	0.20	0.20
Phenol Red	10.00	10.00	10.00	10.00	10.00
Sodium Pyruvate	-	-	-	110.00	110.00
Thymidine	-	-	-	10.00	-
Uridine	-	-	-	10.00	-

Concentration shown in mg/l

List of media components (Continued)

RPMI1640

Nacalai Online Catalog

Product No.	30263	30264	06261	05176	09892
Media	RPMI 1640	RPMI 1640	RPMI 1640	RPMI 1640	RPMI 1640 (No Glucose)
Inorganic Salts:					
Calcium Nitrate・4H_2O	100.00	100.00	100.00	100.00	100.00
Magnesium Sulfate	48.84	48.84	48.84	48.84	48.84
Potassium Chloride	400.00	400.00	400.00	400.00	400.00
Sodium Chloride	5650.00	6000.00	6000.00	6000.00	6000.00
Sodium Hydrogen Carbonate	2000.00	2000.00	2000.00	2000.00	2000.00
di-Sodium Hydrogenphosphate	800.00	800.00	800.00	800.00	800.00
Amino Acids:					
L-Arginine	200.00	200.00	200.00	200.00	200.00
L-Asparagine・H_2O	56.82	56.82	56.82	56.82	56.82
L-Aspartic Acid	20.00	20.00	20.00	20.00	20.00
L-Cystine・2HCl	65.20	65.20	65.20	65.20	65.20
L-Glutamic Acid	20.00	20.00	20.00	20.00	20.00
L-Glutamine	300.00	300.00	300.00	-	300.00
Glycine	10.00	10.00	10.00	10.00	10.00
L-Histidine	15.00	15.00	15.00	15.00	15.00
Hydroxy-L-proline	20.00	20.00	20.00	20.00	20.00
L-Isoleucine	50.00	50.00	50.00	50.00	50.00
L-Leucine	50.00	50.00	50.00	50.00	50.00
L-Lysine・HCl	40.00	40.00	40.00	40.00	40.00
L-Methionine	15.00	15.00	15.00	15.00	15.00
L-Phenylalanine	15.00	15.00	15.00	15.00	15.00
L-Proline	20.00	20.00	20.00	20.00	20.00
L-Serine	30.00	30.00	30.00	30.00	30.00
L-Threonine	20.00	20.00	20.00	20.00	20.00
L-Tryptophan	5.00	5.00	5.00	5.00	5.00
L-Tyrosine・2Na・2H_2O	28.83	28.83	28.83	28.83	28.83
L-Valine	20.00	20.00	20.00	20.00	20.00
Vitamins:					
p-Aminobenzoic Acid	1.00	1.00	1.00	1.00	1.00
D-Biotin	0.20	0.20	0.20	0.20	0.20
Choline Chloride	3.00	3.00	3.00	3.00	3.00
Folic Acid	1.00	1.00	1.00	1.00	1.00
myo-Inositol	35.00	35.00	35.00	35.00	35.00
Nicotinamide	1.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid Calcium Salt	0.25	0.25	0.25	0.25	0.25
Pyridoxine・HCl	1.00	1.00	1.00	1.00	1.00
Vitamin B_1・HCl	1.00	1.00	1.00	1.00	1.00
Vitamin B_2	0.20	0.20	0.20	0.20	0.20
Vitamin B_{12}	0.01	0.005	0.005	0.01	0.005
Others:					
D-Glucose	2000.00	2000.00	2000.00	2000.00	-
Glutathione	1.00	1.00	1.00	1.00	1.00
HEPES	5958.00	-	-	-	-
Phenol Red	5.00	5.00	-	5.00	5.00

Concentration shown in mg/l

Nacalai Online Catalog

● IMDM

● G-MEM

Product No.	11506	12965
Media	IMDM	G-MEM
Inorganic Salts:		
Calcium Chloride	165.00	-
Calcium Chloride, Anhydrous	-	200.00
Iron(III) Nitrate・9H_2O	-	0.10
Magnesium Sulfate	97.67	97.67
Potassium Chloride	330.00	400.00
Potassium Nitrate	0.076	-
Sodium Chloride	4500.00	6400.00
Sodium Dihydrogenphosphate	109.00	-
Sodium Dihydrogenphosphate, Anhydrous	-	107.82
Sodium Hydrogen Carbonate	3024.00	2750.00
Sodium Selenite	0.0173	-
Amino Acids:		
L-Alanine	25.00	-
L-Arginine・HCl	84.00	42.00
L-Asparagine・H_2O	28.40	-
L-Aspartic Acid	30.00	-
L-Cystine・2HCl	91.20	31.00
L-Glutamic Acid	75.00	-
L-Glutamine	584.00	292.00
Glycine	30.00	-
L-Histidine・HCl・H_2O	42.00	21.00
L-Isoleucine	105.00	52.00
L-Leucine	105.00	52.00
L-Lysine・HCl	146.00	73.00
L-Methionine	30.00	15.00
L-Phenylalanine	66.00	33.00
L-Proline	40.00	-
L-Serine	42.00	-
L-Threonine	95.00	47.60
L-Tryptophan	16.00	8.00
L-Tyrosine・2Na・2H_2O	104.00	52.00
L-Valine	94.00	46.80
Vitamins:		
D-Biotin	0.013	-
Choline Chloride	4.00	2.00
Folic Acid	4.00	2.00
myo-Inositol	7.20	3.60
Nicotinamide	4.00	2.00
D-Pantothenic Acid Calcium Salt	4.00	2.00
Pyridoxal・HCl	4.00	2.00
Vitamin B_1・HCl	4.00	2.00
Vitamin B_2	0.40	0.20
Vitamin B_{12}	0.01	-
Others:		
D-Glucose	4500.00	4500.00
HEPES	5958.00	-
Phenol Red	15.00	15.00
Sodium Pyruvate	110.00	-

Concentration shown in mg/l

List of balanced saline solutions

Nacalai Online Catalog

Product Name	Storage	Product No.	PKG Size
D-PBS (-) without Ca and Mg, liquid	RT	14249-95	500 ml
		14249-24	10 x 500 ml
D-PBS (-) without Ca and Mg, liquid (10x)	RT	11482-15	500 ml
D-PBS without Ca and Mg, Powder	RT	07269-84	100 g
D-PBS (+) Preparation Reagent (Ca,Mg Solution) (100x)	RT	02492-94	30 ml
HBSS (+) with Ca, Mg and Phenol Red, liquid	R	17459-55	500 ml
HBSS (+) with Ca, Mg, without Phenol Red, liquid	RT	09735-75	500 ml
HBSS (-) without Ca and Mg, with Phenol Red, liquid	RT	17460-15	500 ml
HBSS (-) without Ca, Mg and Phenol Red, liquid	RT	17461-05	500 ml

List of components for balanced saline solutions

Nacalai Online Catalog

Product No.	02492	14249	17459	09735	17460	17461
Product Name	D-PBS (+) Preparation Reagent (Ca, Mg Solution) (100x)	D-PBS (-) without Ca and Mg, liquid	HBSS(+)with Ca, Mg and Phenol Red,liquid	HBSS (+) with Ca, Mg, without Phenol Red, liquid	HBSS(-)without Ca and Mg, with Phenol Red,liquid	HBSS (-) without Ca, Mg and Phenol Red, liquid
Calcium Chloride	10.00	-	-	-	-	-
Calcium Chloride・$2H_2O$	-	-	184.45	184.45	-	-
D-Glucose	-	-	1000.00	1000.00	1000.00	1000.00
Magnesium Chloride・$6H_2O$	10.00	-	-	-	-	-
Magnesium Sulfate	-	-	97.67	97.67	-	-
Phenol Red, Sodium	-	-	10.20	-	10.20	-
Potassium Chloride	-	200.00	400.00	400.00	400.00	400.00
Potassium Dihydrogenphosphate	-	200.00	60.00	60.00	60.00	60.00
Sodium Chloride	-	8000.00	8000.00	8000.00	8000.00	8000.00
Sodium Hydrogen Carbonate	-	-	350.00	350.00	350.00	350.00
di-Sodium Hydrogenphosphate	-	1150.00	47.88	47.88	47.88	47.88

**Concentration shown in mg/l*

List of supplements

Nacalai Online Catalog

Product Name	Storage	Product No.	PKG Size
L-Alanyl-L-glutamine	R	01102-82	25 g
200mM-L-Alanyl-L-glutamine Solution (100x)	F	04260-64	100 ml
Colcemid Solution(10μg/ml)	R	09356-74	10 ml
200mM-L-Glutamine Stock Solution	F	16948-04	100 ml
1mol/l-HEPES Buffer Solution	R	17557-94	100 ml
MEM Non-Essential Amino Acids Solution (100x)	R	06344-14	20 ml
		06344-56	100 ml
100mM-Sodium Pyruvate Solution (100x)	R	06977-34	100 ml
apo-Transferrin from Human	R	34401-84	100 mg
		34401-55	500 mg

List of cell dissociation reagents

Nacalai Online Catalog

Product Name	Storage	Product No.	PKG Size
2.5g/l-Trypsin Solution	F	35555-54	100 ml
5.0g/l-Trypsin/5.3mmol/l-EDTA Solution	F	35556-44	100 ml
2.5g/l-Trypsin/1mmol/l-EDTA Solution	F	35554-64	100 ml
2.5g/l-Trypsin/1mmol/l-EDTA Solution, with Phenol Red	F	32777-44	100 ml
0.5g/l-Trypsin/0.53mmol/l-EDTA Solution	F	35553-74	100 ml
0.5g/l-Trypsin/0.53mmol/l-EDTA Solution, with Phenol Red	F	32778-34	100 ml
		32778-05	500 ml
0.2g/l-EDTA Solution	R	14367-74	100 ml

List of Antibiotics

Product Name	Application	Mechanism of action	Solvent	Working Concentrations	Storage	Product No.	PKG Size	Online Catalog
Actinomycin D Solution (1mg/ml)	Other Antibiotics	Inhibition of RNA synthesis	Ethanol Ethylene glycol	1 µg/ml	F	00393-41	1 ml	
Antibiotic-Antimycotic Mixed Stock Solution (100x)	Bacteria, Fungal, Yeast	Penicillin: Inhibition of cell wall synthesis Streptomycin: Inhibition of protein synthesis Amphotericin B: Destruction of cell membrane	H_2O Containing 0.85%NaCl	0.01 ml/ml	F	02892-54	100 ml	
Antibiotic-Antimycotic Mixed Stock Solution (100x) (Stabilized)			H_2O		F	09366-44	100 ml	Nacalai Online Catalog
Colcemid Solution (10 µg/ml)	Other Antibiotics	Inhibition of mitosis	PBS	0.05-0.2 µg/ml	R	09356-74	10 ml	
G 418 Disulfate	Selection Antibiotics	Inhibition of protein synthesis	H_2O	50-2,500 µg/ml (Mammalian)	RT	16512-36	250 mg	
						16512-81	1 g	
						16512-94	5 g	
						16512-52	25 g	
G 418 Disulfate Aqueous Solution (50 mg/ml)					R	16513-84	20 ml	
						16513-26	100 ml	
G 418 Disulfate					RT	08973-01	1 g	
						08973-14	5 g	
G 418 Disulfate Aqueous Solution (50 mg/ml)					R	09380-86	20 ml	
						09380-44	100 ml	
Gentamicin Sulfate Solution (10 mg/ml)	Bacteria/ Mycoplasma	Inhibition of protein synthesis	H_2O	50 µg/ml	R	16672-04	10 ml	
Gentamicin Sulfate Solution (50 mg/ml)					R	11980-14	10 ml	
Hygromycin B	Selection Antibiotics	Inhibition of protein synthesis	H_2O HEPES	50-1000µg/ml (Mammalian) 50-100 µg/ml (E.coli)	R	07296-66	100 mg	Nacalai Online Catalog
						07296-11	1 g	
						07296-24	5 g	
Hygromycin B Solution					R	09287-84	20 ml	
Mitomycin C Solution (1 mg/ml)	Other Antibiotics	Inhibition of DNA replication	10% Ethanol 90% Ethylene glycol	10 µg/ml (Induction of Apoptosis)	F	20898-21	1 ml	

List of Antibiotics (Continued)

Product Name	Application	Mechanism of action	Solvent	Working Concentrations	Storage	Product No.	PKG Size	Online Catalog
Penicillin-Streptomycin Mixed Solution Penicillin 10,000 µ/ml, Streptomycin 10,000 µg/ml	Bacteria (Gram-positive bacteria/ Gram-negative bacteria)	Penicillin: Inhibition of cell wall synthesis Steptomycin: Inhibition of protein synthesis	H_2O	0.01 ml/ml	F	26253-84	100 ml	Nacalai Online Catalog
Penicillin-Streptomycin Mixed Solution (Stabilized) Penicillin 10,000 µ/ml, Streptomycin 10,000 µg/ml					F	09367-34	100 ml	
Penicillin-Streptomycin Mixed Solution Penicillin 5,000 µ/ml, Streptomycin 5,000 µg/ml				0.02 ml/ml	F	26252-94	100 ml	
Penicillin-Streptomycin-Glutamine Mixed Solution				0.01 ml/ml	F	06168-34	100 ml	
Streptomycin Sulfate	Gram-negative bacteria	Inhibition of protein synthesis	H_2O	50-100 µg/ml	R	32204-34	5 g	
						32204-92	25 g	

MEMO

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