INTERNATIONAL STANDARD

ISO 24603

First edition
2022-08

Biotechnology — Biobanking — Requirements for human and mouse pluripotent stem cells

Biotechnologie — Biobanking — Exigences relatives aux cellules souches pluripotentes humaines et murines

Reference number
ISO 24603:2022(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have been extensively studied in scientific research in order to improve the understanding of developmental biology and diseases, to create organoids for drug screening, and to be applied in cell-based therapies. In just a few years, thousands of PSC lines have been established in laboratories around the world. PSC lines hold unique characteristics and behaviour due to their capability for both self-renewal and differentiation into multiple cell types. However, the stem cell phenotype can be changed by suboptimal cell culture technique, prolonged passage or changing the culture conditions. Clearly the consequences of using adversely affected cells would be wasted time and resources but, even more seriously, the generation of erroneous data in the literature which could both confuse and delay scientific progress in this area. Accordingly, mouse PSCs have been used to establish our fundamental understanding of PSC biology and these discoveries have been translated into human PSC research to drive the development of new human-cell-based *in vitro* assays and potential regenerative medicines. Mouse PSCs and human PSCs have become the most widely studied species in this field and many significant scientific advances have been made by using PSCs from these two species. Of course, PSC lines have been established from other species such as rat, porcine, canine, bovine, primate, etc. and those from primates in particular have provided understanding of the biology of these cells which can be more relevant to human stem cell biology than data from mouse PSCs. However, PSCs from these species are much less used in research laboratories than mouse and human and are therefore not described specifically in this document although much of this document will be relevant to them.

Human PSCs developed in research environments will give the clues to the development of cell therapies, thus ensuring that cell lines used in this dynamic field have been prepared and documented appropriately and have the correct identity and characteristics, which is critical to help ensure reproducibility in PSC-based research. This document aims to meet the current demand for standardized PSC procedures of biobanks and builds on international consensus agreed by PSC resource centres[9]. This document specifies the establishment, maintenance, characterization, storage and distribution requirements for mouse and human PSCs, providing a general guideline for both biobanking and fundamental research of PSCs.

Biotechnology — Biobanking — Requirements for human and mouse pluripotent stem cells

1 Scope

This document specifies requirements for the biobanking of human and mouse pluripotent stem cells (PSCs), including the collection of biological source material and associated data, establishment, expansion, characterization, quality control (QC), maintenance, preservation, storage, thawing, disposal, distribution and transport.

This document is applicable to all organizations performing biobanking with human and mouse PSCs used for research and development.

This document does not apply to cell lines used for *in vivo* application in humans, clinical applications or therapeutic use.

NOTE International, national or regional regulations or requirements, or multiple of them, can also apply to specific topics covered in this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8601-1, *Date and time — Representations for information interchange — Part 1: Basic rules*

ISO 20387:2018, *Biotechnology — Biobanking — General requirements for biobanking*

ISO/TS 20388:2021, *Biotechnology — Biobanking — Requirements for animal biological material*

ISO 21709:2020, *Biotechnology — Biobanking — Process and quality requirements for establishment, maintenance and characterization of mammalian cell lines*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 20387:2018 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
biobank
legal entity or part of a legal entity that performs *biobanking* (3.2)

[SOURCE: ISO 20387:2018, 3.5]

3.2
biobanking
process of acquisitioning and storing, together with some or all of the activities related to collection, preparation, preservation, testing, analysing and distributing defined biological material as well as related information and data

[SOURCE: ISO 20387:2018, 3.6]

3.3
cell line master file
complete dossier of all procedures and records used to generate and maintain a cell line

3.4
cell morphology
form and structure of the cell

Note 1 to entry: Morphology can be represented by a single parameter or a combination of two or more parameters.

[SOURCE: ISO 21709:2020, 3.3]

3.5
cell population purity
percentage of a particular cell type in a population, of which has the same specific biological characteristics, such as cell surface markers, genetic polymorphisms and biological activities

[SOURCE: ISO/TS 22859:2022, 3.8]

3.6
cryopreservation
process by which cells are maintained in an ultra-low temperature in an inactive state so that they can be revived later

[SOURCE: ISO 21709:2020/Amd 1:2021, 3.6]

3.7
differentiation
process to bring the cells into a defined cell state or fate

[SOURCE: ISO/TS 22859:2022, 3.11]

3.8
differentiation potential
ability that refers to the concept that stem and progenitor cells can produce daughter cells which are able to further differentiate into other cell types

[SOURCE: ISO/TS 22859:2022, 3.12]

3.9
embryonic stem cell
ESC
pluripotent stem cell (3.21) derived from the inner cell mass of a blastocyst, i.e. an early stage pre-implantation embryo

3.10
ethics review committee
body which is responsible for the evaluation and review of the ethical issues involved in the research

3.11
expansion
cell culturing process by which the cell number increases *in vitro*

3.12
feeder cell
mitotically inactivated cell used to support the growth of *pluripotent stem cells* (3.21)

3.13
genetic integrity
genome of cells that has not been altered

3.14
genetic state
phenotype of genetic profile of individual organism, including but not limited to *karyotype* (3.18), integrity, mutation and knock-in of exogenous sequence

3.15
harvest
process of obtaining cells from a cell culture environment

3.16
identity verification
part of the process of verifying authenticity of a cell line in which cell origin is genetically confirmed

[SOURCE: ISO 21709:2020, 3.10]

3.17
induced pluripotent stem cell
iPSC
pluripotent stem cell (3.21) that is generated from somatic cells through artificial reprogramming by the introduction of genes or proteins, or via chemical or drug treatment

3.18
karyotype
characteristics of the chromosomes of a cell, including its number, type, shape and structure, etc.

3.19
passage
subculture
process of further culturing of cells in a culture vessel to provide higher surface area/volume for the cells to grow

Note 1 to entry: A passage can be performed by harvesting an aliquot from the parent vessel and reseeding it into another vessel.

[SOURCE: ISO/TS 22859:2022, 3.18]

3.20
passage number
number of subculturing that occurred

Note 1 to entry: For this document, P_0 is understood as the starting population of the cells.

[SOURCE: ISO 21709:2020, 3.13, modified — Note 1 to entry added.]

3.21
pluripotent stem cell
PSC
stem cell (3.26) that can differentiate into all cell types of the body and is able to self-renew indefinitely *in vitro*

Note 1 to entry: PSCs include *embryonic stem cells (ESCs)* (3.9) (including fertilization derived ESCs, *somatic cell nuclear-transferred stem cells* (3.25), etc.) and *induced pluripotent stem cell (iPSCs)* (3.17).

Note 2 to entry: ESC-like cells can also be isolated by parthenogenetic division of oocytes or other haploid cell sources, and these cells have many of the characteristics of ESCs. However, certain features of these pluripotent cell types can require specific characterization approaches.

3.22
population doubling time
PDT
doubling time
time taken for cultured cell count to double

Note 1 to entry: The time is measured in hours.

[SOURCE: ISO 21709:2020, 3.8, modified — "population doubling time" and "PDT" added as the preferred term. Note 1 to entry added.]

3.23
self-renewal
ability of *stem cells* (3.26) to divide symmetrically, forming two identical daughter stem cells

Note 1 to entry: Adult stem cells can also divide asymmetrically to form one daughter cell which can proceed irreversibly to a differentiated cell lineage and ultimately lead to specialized functional differentiated cells, while the other daughter cell still retains the characteristics of the parental stem cell.

[SOURCE: ISO/TS 22859:2022, 3.23]

3.24
separation
process of obtaining target cells from biological samples

3.25
somatic cell nuclear-transferred stem cell
embryonic stem cells (3.9) derived from *in vitro* transfer of a donor cell nucleus into an enucleated oocyte

3.26
stem cell
non-specialized cells with the capacity for *self-renewal* (3.23) and *differentiation potential* (3.8), which can differentiate into one or more different types of specialized cells

Note 1 to entry: Based on potency, stem cells can be divided into: *totipotent stem cell* (3.29), *pluripotent stem cell* (3.21), multipotent stem cell, oligopotent stem cells, and unipotent stem cells (see Annex A).

[SOURCE: ISO/TS 22859:2022, 3.24, modified — Note 1 to entry replaced.]

3.27
stem cell marker
protein or gene specifically expressed in *stem cells* (3.26), usually used to isolate and identify stem cells

Note 1 to entry: Stem cell markers vary depending on stem cell type.

3.28
teratoma
tumour containing representative differentiated tissues and cells from the three germ layers

3.29
totipotent stem cell
stem cell (3.26) that can differentiate into an intact new organism including embryonal and extra embryonal cells

3.30
viability
attribute of being alive (e.g., metabolically active, capable of reproducing, have intact cell membrane, or have the capacity to resume these functions)

[SOURCE: ISO 21709:2020, 3.17, modified — "as defined based on the intended use" deleted.]

4 Abbreviated terms

bFGF	basic fibroblast growth factor
EMRO	embryo research oversight
ESC	embryonic stem cell
HBV	hepatitis B virus
HCMV	human cytomegalovirus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HTLV	human T-lymphotropic virus
IFU	instructions for use
iPSC	induced pluripotent stem cell
KLF4	krueppel-like factor 4
KSR	knockout serum replacement
mLIF	mouse leukaemia inhibitory factor
MTA	materials transfer agreement
OCT4	octamer-binding transcription factor 4
OriP	origin of replication
PBMC	peripheral blood mononuclear cell
PSC	pluripotent stem cell
QC	quality control
SOX2	SRY (sex determining region Y)-box 2
SSEA3	stage-specific embryonic antigen 3
SSEA4	stage-specific embryonic antigen 4
SSEA1	stage-specific embryonic antigen 1
STR	short tandem repeat
SV40LT	Simian virus 40 large T

TP treponema pallidum

5 General requirements

5.1 General

The biobank shall follow ISO 20387 and ISO 21709, in addition to this document. ISO/TR 22758 can be used as additional reference for the implementation of ISO 20387. For mouse PSCs, ISO/TS 20388 shall also be followed.

The biobank shall establish criteria and procedures for the isolation, establishment, expansion, storage, thawing and transport of PSCs.

A data analysis procedure shall be established, documented, implemented, regularly reviewed and updated.

The biobank shall use validated and/or verified methods and procedures for activities pertaining to PSCs in accordance with ISO 20387:2018, 7.9.2 and 7.9.3, at all stages of the biological material life cycle (as defined in ISO 20387:2018, 3.29).

According to the characteristics of PSCs, procedures, QC documents for collection, separation, expansion, storage, transportation and testing, and data analysis shall be established, documented, implemented, regularly reviewed and updated.

5.2 Legal and ethical requirements

ISO 20387:2018, 4.1.6, 4.3, 7.2.3.4, 7.3.2.4, A.7 a), and ISO 21709:2020, 4.2, shall be followed. For mouse PSCs, ISO/TS 20388:2021, 4.2, shall also be followed.

The biobank shall collect relevant information on ethical requirements, implement and regularly update them, where relevant.

It is important to recognize that PSC lines are potentially not acceptable for use in research or development or both in some countries, and shipment of cells to collaborating organizations will require consideration of these differences. The biobank shall establish, document and implement policies on the procurement and supply of PSCs.

Experimental plans using or establishing human PSCs should be consulted in a specialized ethics review committee with particular expertise in topics relevant to the type and intended use of the PSC lines in the biobank.

The biobank shall establish a process to verify and document cell line provenance, to be able to provide evidence of ethical and regulatory compliance.

The biobank shall be aware whether reimbursement was made for the donation of human embryos/tissues and whether the human embryo was created for research as this can be illegal in some countries.

For derivation of new pluripotent cell lines from human embryos, the ethical review process shall refer to relevant expert ethical reviews.

EXAMPLE The human embryo research oversight (EMRO) process (ISSCR guidelines 2016, Chapter 2.1)[10].

Ethical requirements relevant for distribution are provided in 18.1.

5.3 Personnel, facilities and equipment

ISO 20387:2018, Clause 6, and ISO 21709:2020, 4.3, 4.4, 4.7, shall be followed. For mouse PSCs, ISO/TS 20388:2021, 4.3, shall also be followed.

The biobank personnel shall be appropriately and specifically trained in PSC generation, characterization, culture, cryopreservation, thawing and transport.

The biobank shall ensure that external operators providing PSC services demonstrate relevant knowledge, experience and corresponding skills.

The biobank shall ensure that facilities, equipment and environmental conditions do not adversely affect PSC quality attributes or invalidate the test results.

Equipment management procedures should be established, including the use of equipment and maintenance plan.

The biobank shall control the operating environment and conditions (e.g. temperature, humidity, cleanliness) according to the relevant characteristics of PSCs and the need for aseptic processing.

5.4 Reagents, consumables and other supplies

ISO 21709:2020, 4.5, shall be followed. For mouse PSCs, ISO/TS 20388:2021, 5.1.3, shall also be followed.

The biobank shall establish acceptance criteria for materials, including reagents and consumables, necessary for PSC isolation, establishment, expansion, preservation, storage, thawing and transport.

If animal serum is used for PSC culture, there should be no evident potential high risk source of virus or bovine spongiform encephalopathy, which cannot be managed by a risk assessment of the biological source material and decontamination (such as irradiation for certain viruses).

For culture of human cell lines, if there are blood components in the culture medium (such as platelet lysate, serum, albumin, transferrin and various cytokines), the source, batch number and quality verification report shall be documented; and, if possible, a risk assessment shall be completed following communication with the manufacturer on the risk of microbial contamination and other potential hazards such as toxic contaminants. Where approved sources of these components are available, they shall be used unless unsuitable for technical or logistical reasons.

5.5 Management of information and data

ISO 20387:2018, 7.8.3 and 7.10, shall be followed.

The biobank shall manage and maintain associated data of PSC lines, including but not limited to the following:

a) the technical information: methods used in the derivation of cells/lines, culture conditions, passage data including the passage number, characterization and microbiological test data;

b) the preservation and storage information;

c) the safety testing data;

d) the cell identity verification methods, e.g. by short tandem repeat (STR) analysis and/or HLA-typing or equivalent validated methods.

Certain data retention times, data integrity and security of data storage shall be ensured.

For human PSCs, a minimum period of retention of records shall be established. Special requirements for storage and retention times can apply for future applications. Personal data of each human donor shall be held in a protected location and shall be handled in accordance with ISO 20387:2018, 4.3.

The cell line master file shall be kept to enable review of the data and records for specific applications.

6 Collection of biological source materials and associated data

6.1 Information about the human donor and requirements for the biological material

A risk assessment shall be performed and documented.

To protect the private data of the donor, the biobank shall establish donor data protection methods in accordance with ISO 20387:2018, 4.3.

The documentation of the donor information shall be performed. Where possible, the documentation shall be performed prior to sample collection. The documentation shall include but is not limited to:

a) the donor reference, which can be in form of a code (e.g. pseudonymized, anonymized);

b) the relevant health status of the donor (e.g. medical history, statement of donor health or suitability, disease type, concomitant disease, demographics such as age and sex);

NOTE The ABO blood groups and category classification data of HLA of the donor can also be collected depending on the situation.

c) the information about medical treatment and special treatment prior to the collection (e.g. date, terms of treatment, medication, conclusion of medical specialist);

d) where applicable, information about the informed consent given by the donor (e.g. copy of the signed informed consent signature form with details of the donors' name redacted); see ISO 20387:2018, 7.2.3.4.

Documentation of the donor information should include the geographical region of the donor as needed based on the purpose of research.

For planned iPSC line establishment, the documentation of donor information shall include but is not limited to:

— sex;

— age;

— tissue or cell type.

During the collection process for human cells, measures shall be taken to protect donor and biobank personnel health and safety.

6.2 Information about the mouse donor and requirements for the biological material

The biobank shall establish, document and implement inclusion and exclusion criteria based on the purpose of research.

Documentation of the mouse donor information shall take place prior to collection, shall include a) and b), and should include, but is not limited to c) and d) of the following list:

a) the strain and genotype;

b) the demographics (i.e. age and sex, etc.);

c) the relevant health status of the mouse (e.g. statement of donor health or suitability, disease type, concomitant disease);

d) the information about medical treatment and special treatment prior to the collection (e.g. date, terms of treatment, medication, conclusion of medical specialist, stress, diet).

The ID of the mouse, which can be in form of a code (e.g. according to Reference [11]), should be additionally documented, if available. For planned iPSC line establishment, the documentation of donor information shall additionally include but is not limited to:

— sex;

— age;

— tissue or cell types;

— the reprogramming strategies including reprogramming factors and gene delivery system.

The animal welfare requirements of donor mouse husbandry should conform to ISO 10993-2:2006.

If the mouse PSC is established from a specific strain or genetic modified mouse developed by another laboratory, company or organization, an agreement for new established mouse PSCs shall be required with the mouse donor's legally designated representative.

If the donated mouse is genetically modified, its use does not necessarily conflict with the original owner's rights.

For PSCs from mice, any known colony infectious agent screening shall be documented.

6.3 Collection procedure

ISO 20387:2018, 7.2, shall be followed.

The biobank shall establish, implement, validate and document a collection procedure for each relevant biological source material.

NOTE Each selected tissue has specific requirements for collection and best practice. Taking into account new developments can improve the quality of harvested cells.

All reagents and materials used to collect the biological material shall be sterile.

The biobank should conform to ISO 35001 or the WHO's *Laboratory Biosafety Manual*[12] when handling biological material contaminated with pathogens.

The risk of microbiological contamination (bacterial, fungal, viral, parasitic) should be mitigated by focusing on those agents which are most likely to be contaminants in relation to the geography, donor cohort and tissue being procured.

7 Transport of the biological source material or PSCs and associated data to the biobank

ISO 20387:2018, 7.4, shall be followed. ISO/TS 20658 can be used to consider transport and handling, and safety requirements for facilities. For mouse PSCs, ISO/TS 20388:2021, Clause 6, shall also be followed.

The biobank should conform to ISO 35001 or the WHO's *Laboratory Biosafety Manual*[12] when handling biological material contaminated with pathogens.

The biobank shall determine the appropriate conditions for the transportation of the biological source material from the collection facility to the biobank. Instructions on the transportation of biological source material to the preparation site as well as the transportation of PSC preparations to the biobank should be included.

The following factors shall be taken into account for transportation:

a) packaging, material, containers and secondary containment;

b) medium or solvent;

c) transportation duration and temperature.

Biological source material storage media and conditions shall be established, implemented, documented and validated to ensure maintenance of the viability and other key parameters.

The sample shall be transported under appropriate biosafety conditions.

A procedure for critical control points shall be established, implemented and documented.

8 Reception and traceability of the biological source material or PSCs and associated data

ISO 20387:2018, 7.3.1, 7.3.2 and 7.5, shall be followed. For mouse PSCs, ISO/TS 20388:2021, Clause 7, shall also be followed.

9 Establishment of cell lines

9.1 Processes

For establishing human PSC and mouse PSC lines, ISO 21709:2020, 5.1, shall be followed. Examples of suitable methods for the establishment and culture of PCS lines are given in Annex B.

The biobank shall establish, implement, validate, document and maintain procedures for isolation and primary culture of relevant cell lines.

Processes should be performed in a biosafety cabinet or under a laminar flow hood using appropriate aseptic techniques.

For iPSC line establishment, the reprogramming strategies including reprogramming factors and gene delivery system shall be clearly documented.

Each culture expansion is referred to as a "subculture" or "passage".

9.2 Unique identification

The unique identification of PSC lines shall be established in accordance with ISO 20387:2018, 7.5. This should include a unique cell line name (such as that generated by registration in the hPSCreg®[1)] database[13] for human PSCs), or sample number, a biobank batch number and biobank vial number. Cell lines should be anonymized or de-identified.

9.3 Testing for infectious agents

The human donor biological material or the cell line(s) derived from this material should be tested for relevant transmittable infectious agents, e.g. HIV, HBV, HCV, HTLV, HCMV and TP. A report regarding the condition of the mouse donor, including information on results of specific pathogen testing, shall be obtained from the provider.

The analytical data and results as well as the associated analyses shall be documented and available to authorized biobank personnel and researchers who process biological material and established cell lines.

1) hPSCreg® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

10 Characterization

10.1 General

The biobank shall establish, document and implement procedures to characterize PSCs and report the relevant data so that users can determine suitability for their intended use.

Biological characteristics of PSC lines shall be established by the biobank in accordance with current scientific best practice and international consensus (e.g. Reference [14]). The characterization shall include but is not limited to the following:

a) Cell morphology: Cells grow under 2D conditions and typically exhibit growth as colonies with clear colony boundaries, high nuclear-cytoplasmic ratio and uniform morphology. Within the clone, cell-cell contact is tight.

b) Cell identification: Cell lines have a unique donor genetic profile and this profile can be used to facilitate exclusion of cross-contamination with other cells and confirm donor origin. The requirements on the cell line authentication can refer to ISO/TS 23511[2]. Biological source materials for establishing PSC lines or the PSC in early passage shall be used where possible to establish the initial STR profile as a reference for subsequent STR profiling, e.g. to check for cross-contamination or cell identity.

c) Genetic integrity: Chromosome karyotype analysis: For cells from non-disease affected donors, these are typically as follows: human PSCs: 46, XX or 46, XY; mouse PSCs: 40, XX or 40, XY.

   For biobanks of PSC lines, karyological variants can arise and in such cases the potential impact on cell line performance should be assessed either by the biobank or by users of the biobank.

d) Cell viability: A range of viability assays can be used and each measures a different aspect of cell biology. Such tests include cell metabolic activity [the function of esterase, Thiazole blue method based on the determination of succinic dehydrogenase (MTT also known as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), apoptosis markers, cell redox potential, membrane potential, proliferation rate (DNA content), mitochondrial function and membrane integrity].

   NOTE 1 Cell viability is addressed in ISO 23033:2021, 7.5.

e) Stem cell markers: PSCs express cell markers including but not limited to self-renewal markers (e.g. OCT4, SOX2, NANOG in both human PSCs and mouse PSCs) and canonical markers indicative of pluripotency (e.g. human PSC: TRA-1-81, TRA-1-60, SSEA3, SSEA4; mouse PSC: SSEA1). The expression level of these markers can vary in different culture conditions. The stated markers shall be present in the majority of the cells, e.g. by immunofluorescence and/or gene expression analysis. The operator should establish cut-off values fit for purpose.

f) Pluripotency assay: A range of assays can be employed to reveal the pluripotency of PSCs (see Table 1):

   1) *in vitro* differentiation: induction of embryoid bodies or directed differentiation to the cell types representative of each germ layer.

      NOTE 2 Some cell lines, while failing the test, can be useful, e.g. for research to understand the defect and for preparation of a particular type of differentiated cells.

   2) Teratoma formation: Assessing the spontaneous generation of differentiated tissues from the three germ layers following the injection of PSCs into immune-compromised mice.

      NOTE 3 Some cells with incomplete pluripotency potential can also generate masses that superficially resemble teratomas yet lack terminal three-germ-layer differentiation, potentially leading to misinterpretation.

2) Under preparation. Stage at the time of publication: ISO/DTS 23511.2:2022.

NOTE 4 The teratoma assay is currently widely used and is considered as the gold standard functional assay for assessing human PSC developmental potential. Due to ethical and legal restrictions, other human PSC pluripotency assays *in vivo* (such as chimaera formation and tetraploid complementation) cannot be performed.

NOTE 5 Teratomas are not generated from single cells, the teratoma assay assesses developmental potency at a population-based level.

3) for mouse cell lines, chimaera formation: assessing whether cells can re-enter development when introduced into host embryos at either morula or blastocyst stages.

NOTE 6 Human xenogenics are outside of the scope of this document.

NOTE 7 PSCs of high quality can support normal development and generate high-grade chimaeras with extensive colonization of all embryonic tissues including the germ line, whereas less-potent PSCs produce either low chimaerism or reduced embryo viability.

4) Germline transmission: assessing whether the test PSCs can generate functional gametes by breeding chimaeras to produce all-donor PSC-derived offspring.

NOTE 8 This is prohibited in human PSC pluripotency assessing, see the international stem cell research guidelines[10].

5) Tetraploid complementation: assessing whether the test PSCs can direct development of an entire organism by introducing donor PSCs into tetraploid (4n) host blastocysts, which can be generated by electrofusion of blastomeres at the two cell stage. The 4n blastocysts cannot sustain normal embryonic development beyond mid-gestation, while tetraploid extra-embryonic tissues develop normally and support donor cells. Any resulting embryos are derived essentially entirely from donor PSCs. The ability to perform such tests can be restricted in certain countries varies and, where not feasible, alternative validated *in vitro* assays can be used.

NOTE 9 Tetraploid complementation is prohibited in human PSC pluripotency assessing, see the international stem cell research guidelines[10].

g) In the case of reprogrammed iPSCs, the ongoing expression of reprogramming factors from the reprogramming vectors used can affect the ability of an iPSC line to differentiate properly or efficiently. The elimination of ectopic expression of the reprogramming factors shall be confirmed and documented.

Table 1 — Pluripotency assay

	***in vitro* differentiation**	**teratoma formation**	**chimaera formation**	**germline transmission**	**tetraploid complementation**
functional stringency	+	++	+++	++++	+++++

10.2 Population doubling time and subculture/passage

10.2.1 PDT

The PDT is the time (measured in hours) required for the replication of the population of PSCs. The PDT is calculated with Formula (1) using the cell counts obtained before and after harvesting:

$$D = (T - T_0) \times \log^2 / (\log N - \log N_0) \quad (1)$$

where

D is the PDT;

$(T - T_0)$ is the incubation time in hours;

N is the count of cells harvested;

N_0 is the count of cells seeded.

NOTE 1 Formula (1) is applicable in a linear range of cell expansion.

The average PDT of mouse PSCs ranges typically between 11 h and 20 h.[15][16][17][18] The average PDT of human PSCs ranges between 16 h and 48 h[16][19][20][21].

NOTE 2 Depending on the culture conditions, culture passage, cell density and characteristics of the donor (e.g. age), the PDT can vary.

The PDT of PSCs should be determined by the biobank after secondary culture.

PDT can reflect the growth kinetics of PSCs in culture. The biobank can utilize the PDT of PSC cultures at different passages to evaluate changes in culture cell growth kinetics.

The PDT shall be documented.

10.2.2 Subculture/passage

P_0, the passage number(s) together with the seeding and final cell density, and the culture vessel surface area shall be documented. When the PSCs cover the culture vessel surface at 70 % to 80 %, the cells can be passaged.

Passage numbers are frequently used by laboratories. However, the passage number is correlated with the surface area/volume of a culture vessel and how the initial P_0 is defined. It is recommended that the biobank defines P_0 as the initial plating of cells.

Documenting PDT along with passage numbers can facilitate a better understanding of growth dynamics of the PSCs and the relationship between passages and PDT.

10.3 Stability of the culture

Stability indicators of PSCs shall be established, implemented and documented by the biobank according to the intended purpose, including but not limited to trends of:

a) the density/concentration;

b) the cell population purity;

c) the survival rate;

d) the genetic state/integrity;

EXAMPLE As determined by karyotyping, single nucleotide polymorphism arrays, whole genome sequencing, etc.

e) the biological activity of the PSCs.

Some degree in variation of the PSC lines is expected, but progressive or irreversible changes, such as changes in the genetic sequence, chromosome changes, growth rate or morphology, should be documented and assessed to explore any potential deleterious impact on the PSCs' pluripotent properties or other effects which can impair the quality of differentiated cells.

There has been considerable debate about the most appropriate test for genetic stability. The biobank shall decide on the features of stability that it needs to monitor and maintain awareness of them based on the latest research consensus regarding the most suitable methodologies to use.

10.4 Functionality

10.4.1 General

The functionality indicators of PSCs shall be established according to the intended application of the cells, which include but are not limited to differentiation potential, the structure and physiological function of the differentiated cells, and the expression of specific genes and proteins, as well as the secretion of specific cellular factors of PSCs, etc.

For human PSCs, it is especially important to pay significant attention to such tests as they will provide a basis for critical assays that need to be defined to reach the appropriate fitness for the intended research purpose and can involve additional biomarkers to those used in standard QC and characterization for cells intended for broad research purposes.

10.4.2 *In vitro* differentiation

The starting cells, equipment, culture system and operation procedures used in cell differentiation shall be documented.

The characterization of the differentiated cells generated during the differentiation of PSCs should not be limited to specific morphological features. The functional identification should also be established, such as the expression of the crucial system markers. The selection of these system markers requires careful consideration and validation.

Experiments involving human blastocyst *in vitro* development using PSCs should not exceed 14 days or as otherwise stated in the respective materials transfer agreement (see 18.1). The biobank shall be aware whether the period for experiments involving human blastocyst *in vitro* development using PSCs is determined by law or regulation of the relevant region(s) or countries(s).

10.5 Microbial contamination

Procedures for microbial contaminant testing of PSCs shall be established, validated, implemented and documented throughout the whole process.

Throughout the whole process from donation and procurement, preparation of culture reagents and equipment, to maintenance and cryopreservation of cultures, it is important to take a holistic view and establish microbiological testing at all critical points of the process. In addition, procedures to minimize risks to other established cultures should be in place. It is good practice to maintain QC procedures for primary tissues or cells newly brought into the biobank. Such cultures should be maintained in a dedicated area and in segregated equipment until sufficient data are available to justify their relocation.

Test methods used for microbiological testing shall be validated. It is important to be sure that appropriate levels of sensitivity, specificity and robustness are being used in respect of testing cell cultures.

Microbial contamination shall be assessed by risk management throughout the process.

11 Quality control

ISO 20387:2018, 7.8, and ISO 21709:2020, 5.5, shall be followed. For mouse PSCs, ISO/TS 20388:2021, 8.5, shall also be followed.

The biobank shall establish, implement and document a QC procedure, which shall include the testing of biological characteristics related to the *in vitro* functionality of PSCs as given in Clause 10.

QC of biological characteristics (see Clause 10) of PSCs shall be performed for all critical procedures, from isolation to thawing. An exemplary QC procedure for biobanking of PSCs is given in Annex C.

The biobank shall establish, implement and document QC acceptance criteria for all the biological characteristics of PCSs included in Clause 10.

The biobank shall establish, implement and document QC acceptance criteria for all critical control points, e.g. culture media, reagents, equipment.

QC shall be established with a risk-based approach related to laboratory safety.

Safety indicators of PSCs shall be established by the biobank according to the application, which addresses both microbiological risk and cell-derived risk.

Microbiological testing, including but not limited to sterility testing (bacterial, fungus), mycoplasma testing and endotoxin testing, shall be performed:

a) If required by the user/biobank, human PSCs shall be tested for relevant fungi, bacteria, infectious agents (e.g. HIV, HBV, HCV, HTLV, HCMV, TP) and mycoplasma by validated methods for cell cultures. Risk assessment on the donor and cell culture reagents can also reveal that additional testing for microorganisms needs to be performed. PSC should be negative for fungi, bacteria and mycoplasma.

b) Mouse PSCs should be tested negative for fungi, bacteria and mycoplasma, and should not show overt signs of viral cytopathic effect. Risk assessment on the donor animals and cell culture reagents can also reveal additional testing that can be needed to be performed for microorganisms. Mouse colonies used to derive PSCs should be subject to microbiological screening according to animal husbandry best practice.

Cell-derived hazard testing, including but not limited to testing of pathogenic internal and external cellular factors, abnormal immunological response, tumorigenicity and genetic stability, should be performed depending on the potential ultimate future use of the cells.

12 Testing

Relevant testing procedures shall be developed, implemented and documented to ensure the accuracy and reliability of the testing processes and test results.

For testing equipment and relevant facilities/environment, see 5.3.

13 Cell line management

The biobank shall sustain a master and distribution PSC biobanking system[22] to ensure consistent supplies of cell lines to users over time[9].

14 Preservation of cell lines

ISO 20387:2018, 7.6, shall be followed.

Documentation shall be performed for each batch of preserved cells.

In the process of cell expansion, the potential for cross-contamination and switching of cell lines shall be minimized by a combination of documentation and procedural controls, and suitable testing implemented to expose non-authentic cell lines and incidences of cell line cross-contamination during cell biobanking procedures. Such analysis should facilitate comparison of cell lines banked in the same biobank and a DNA fingerprint of each cell line compared to DNA from the donor of origin.

During the process of PSC expansion the culture generation (by recording cell number and viability at harvest and seeding of new cultures), cell line name, the operation date, culture conditions, operator name or initials and any protocol deviations and corrective actions shall be documented.

15 Storage

ISO 20387:2018, 7.5, 7.7 and Clause A.6, and ISO 21709:2020, 5.3.4, shall be followed.

Optimization of the cryopreservation procedure and method(s) to minimize damage to cells during freezing and thawing is critical to ensure reliable availability of viable cells.

NOTE Controlling the freezing rate, using an appropriate cryoprotectant, and maintaining a stable storage temperature can minimize negative effects on the cell viability.

For cryopreserved PSCs, the following information shall be documented:

a) the cell name;

b) the preserved PSC batch number;

c) the date of preservation in accordance with ISO 8601-1;

d) the culture conditions;

e) the passage number;

f) the operator name.

Each stored vial derived from the same batch of cultured cells shall have a unique identification reference number (i.e. a biobank or batch number), which is traceable throughout the processes of collection, separation and expansion in accordance with ISO 20387:2018, 7.5.

The biobank shall maintain records of the cryopreservation process, including the cell density, viability and temperature control.

16 Thawing

In the cell thawing process, frozen cells shall be thawed at 37 °C ± 0,5 °C, processed for culture, put into culture and then transferred into an incubator with appropriate gas atmosphere and humidity. To optimize the process, the incubator shall be set to an appropriate culture temperature, which is typically 37 °C ± 0,5 °C.

The frozen cells should be quickly thawed by warming and transferred directly to pre-warmed culture medium at 37 °C ± 0,5 °C to ensure maximal PSC viability and biological activity.

The following information should be documented, including but not limited to:

a) the batch number of the set of frozen vials;

b) the cell name;

c) the passage number;

d) the culture condition;

e) the operator name;

f) the thawing date of the thaw operation in accordance with ISO 8601-1;

g) the thawing time in accordance with ISO 8601-1 as the time point when the frozen cells leave liquid nitrogen to the time point when the cells are put into culture;

h) the date in accordance with ISO 8601-1 at which the culture reaches sufficient colony density to be passaged.

Cell viability shall be tested after thawing.

17 Disposal

For managing waste disposal, ISO 20387:2018, 4.1.8, 7.1.1, 7.5.3, 8.4.2, Clause A.7, and ISO 21709:2020, 5.3.6, shall be followed.

Any disposal of PSCs (e.g. embryo, germ cell, bone marrow, blood) shall be conducted in accordance with applicable environmental, biosafety and ethical requirements.

18 Distribution

18.1 General requirements

ISO 20387:2018, 7.3.3, and ISO 21709:2020, 5.4, shall be followed. For mouse PSCs, ISO/TS 20388:2021, 10.1, shall also be followed.

Depositors of cells shall provide documented evidence that demonstrates they have met all their national legal and ethical requirements associated with procurement of tissue and derivation of the cell lines.

If the organization or the individual wants to apply for PSCs, they shall submit an application and be approved by the PSCs storage organization in accordance with its formal written procedures.

Only ethically approved projects shall receive cells from the biobank. This can be assessed by the biobank or an ethics review group.

A materials transfer agreement (MTA) or an equivalent terms and conditions document shall be signed by the recipient of the cells and stored by the biobank. The MTA content should prevent distribution for unethical purposes and prohibit third-party distribution or change of research project without prior permission from the biobank. The biobank should take into account the inclusion of MTA clauses prohibiting reproductive cloning that uses human PSCs.

A common MTA is not necessarily practicable for all biobanks. However, biological resource organizations have identified key generic elements that should be included (e.g. European Culture Collections' Organisation[23]) and there are other national examples that can be considered as templates (e.g. National Cancer Institute, see also links to national biobanks on the International Stem Cell Forum (ISCF) website to obtain MTAs from suppliers of stem cell lines).

The MTA should stipulate the rights and responsibilities of the biobank and the recipient, including requirements for any transfer to third parties.

Culture manuals should be available from the biobank, ideally online, including key standard operating procedures. The release of cells to users should be accompanied with advice and training. Users should either have evidence of past training or training should be provided as part of cell supply. Minimum instructions for users shall contain protocols for thawing of the cryopreserved cells.

A complaint procedure shall be established, implemented and documented in accordance with ISO 20387:2018, 7.13. The biobank shall have a replacement policy for cultures which fail to thrive in the hands of user(s). All complaints should be reviewed to assess the effectiveness of the corrective actions taken and to look for opportunities to improve service.

18.2 Information for users

ISO 20387:2018, 7.12, shall be followed.

Instructions for use (IFU) and/or standard operational procedures for isolation, expansion, preservation, storage and transport of PSCs should be provided to biobank users. The IFU should typically contain information prescribing general culture and preservation methods and what procedures the cells have been qualified or consented for (e.g. "*in vitro* research only", "not for generation of gametes", "not for reproductive cloning").

Batch numbers, traceable to the batch or biobank, and a statement or material safety data sheet on hazards for the cells shipped shall be provided to users.

Terms and conditions or a warranty, which qualifies cell potential and characteristics based on testing performed by the biobank, should be provided.

The characterization and microbiological test data from the depositor for cell line in the biobank shall be available for bank users.

The biobank should have a documented policy for the quality and sourcing of raw materials that can impact the quality of cell preparations, subject to national or international restrictions, e.g. fetal bovine serum, trypsin, growth factors.

The biobank should provide information to facilitate the efficient selection of suitable cell lines. Information should include but is not limited to:

a) the date of collection and preservation of tissue in accordance with ISO 8601-1, if available;

b) the date, in accordance with ISO 8601-1, of attempted derivation (for human ESCs, this is usually considered to be the date the inner cell mass was isolated or plated *in vitro*);

c) whether fresh or frozen embryos has been used;

d) where applicable, relevant information regarding informed consent obtained from the human donor for use of the original tissue for research;

e) where applicable, relevant information regarding the necessary animal welfare approval(s);

f) any associated constraints on the use of the derived cell line;

g) the data and interpretation resulting from characterization and QC.

18.3 Transport

18.3.1 ISO 20387:2018, 7.4, and ISO 21709:2020, 5.4.4, shall be followed.

According to the requirements of the use of PSCs, the appropriate mode of transportation and transportation conditions shall be selected to enable maintenance of the biological characteristics, safety, stability and viability of PSCs.

The biobank shall establish, implement and document procedures for the transport and handling of PSCs and their associated data.

Unnecessary exposure to radiation should be avoided during shipment.

PSCs can be transported as frozen ampoules/vials or as living cultures; in either case:

a) advise the recipient as to when the cells are to be shipped;

b) provide written instructions on the following:

   1) instructions upon reception of PSCs;

   2) instruction for thawing and reconstitution of PSCs;

   3) instructions for secondary storage conditions;

   4) medium or serum required;

   5) any special supplements;

6) subculture regimen;

c) tape the cells' data sheet and a copy of the instructions to the outside of the package so that the recipient knows what to do before opening it.

Each frozen ampoule/vial or living culture container (primary container) can be introduced into a pre-sterilized self-adhesive seal package. The packaging shall be labelled with:

— the sample's data;

— the production and expiration date;

— the name and contact information of the entity that performs biobanking.

18.3.2 The transport of PSCs should be qualified by the biobank and accepted by the cell requestor(s). The qualification should include but is not limited to:

a) the container carrying cells;

b) the transportation routes;

c) the transportation conditions and maximum shipment duration;

d) the transportation equipment and transportation methods;

e) the transportation risks;

f) the safeguard measures.

18.3.3 The biobank shall establish and control transportation conditions such as:

a) the temperature range;

b) the vibration in case of transporting living PSCs;

c) the contamination prevention;

d) the validation of the equipment performance of the shipment device (e.g. dry-shipper, vials containing the sample);

e) the appropriate packaging;

f) the directional placement of the sample;

g) the allowed radiation;

h) the humidity when transporting living PSCs.

NOTE This can be verified by online monitoring or transport process control.

18.3.4 The transport of PSCs shall be documented including but not limited to:

a) the mode and condition of the PSCs transportation;

b) the route of transportation;

c) the shipping agent and their contacts;

d) the recipient details (i.e. person, address and other information).

18.3.5 Where necessary, arrangements should be made to check packages in transit and new cryogens (e.g. solid carbon dioxide, liquid nitrogen) added, where possible, to maintain the appropriate shipping temperature.

18.3.6 With current culture methods, biobanks should avoid shipment of growing cultures. Pooling multiple straws or vials from the same frozen stock can be necessary for a recipient to successfully thaw a culture. However, biobanks should aim to provide sufficient viable cells in a single container to enable appropriately trained staff to thaw a representative culture.

Annex A
(informative)

Potency comparison

Table A.1 — Potency comparison

	Totipotent	Pluripotent	Multipotent	Oligopotent	Unipotent
Potency	+++++	++++	+++	++	+
Terminology	Toti = whole	Pluri = many	Multi = several	Oligo = a few	Uni = single
Cell types capable of generating	Differentiate into any cell type as well as extra-embryonic cells (placenta)	Differentiate into cells from any of the three germ layers (but not the placenta)	Differentiate into a limited number of cell types in a particular lineage	Differentiate into a few types of cells	Differentiate into only one type of cells
Examples	Zygote	Embryonic stem cells, induced pluripotent stem cells	Haematopoietic stem cells, neural stem cells, mesenchymal stem cells	Myeloid stem cells	Myoblast

Annex B
(informative)

Examples for methods for the establishment and culture of PSCs

B.1 General

There are varieties of methods for the establishment and passage of human and mouse PSCs. The approaches given in B.2 can be taken into consideration as options.

B.2 Process

B.2.1 Human ESC establishment

Information for approaches for the establishment of human ESCs can be found in References [24] and [25].

B.2.2 Human iPSC establishment

B.2.2.1 Episomal reprogramming method

Feeder-free iPSC derivation from human neonatal foreskin fibroblasts is used as an example.

a) Episomal vectors: There are different versions of episomal vectors currently available. The type of episomal vectors routinely used for human iPSC derivation is based on OriP/EBNA1 elements from Epstein-Barr virus.

b) Reprogramming factors: Many different combinations of reprogramming factors can be used, e.g. human OCT4, SOX2, NANOG, LIN28, L-MYC, KLF4 and SV40LT. These genes are cloned in appropriate expression cassettes in episomal vectors.

c) Culture human neonatal foreskin fibroblasts in appropriate medium, e.g. DMEM supplemented with 10 % heat-inactivated fetal bovine serum, 0,1 mM non-essential amino acids, 1 mM GlutaMAX, 0,1 mM β-mercaptoethanol and 4 ng/ml bFGF. Transfect episomal reprogramming vectors into foreskin fibroblasts either chemically or via electroporation. To improve transfection efficiency, nucleofection can be performed.

d) Plate transfected fibroblasts at low cell density in fibroblast culture medium. The surface can be precoated with matrix such as Matrigel, Vitronectin or Laminin 511-E8. Low cell-density plating is critical here, e.g. ~1,0 × 10^6 nucleofected fibroblasts can be plated into three 10 cm dishes.

e) Culture transfected fibroblasts in reprogramming medium. To improve feeder-free episomal reprogramming efficiency, the reprogramming culture can be divided into two stages. During the first stage (e.g. day 1 to day 13 for neonatal foreskin fibroblasts), serum-free fibroblast culture medium (e.g. N2B27 basal medium) supplements with small molecules (e.g. A-83-01, PD0325901, CHIR99021, HA-100) and growth factors (e.g. LIF, bFGF) can be used. During the second stage (e.g. day 13 to day 25), appropriate feeder-free human ESC culture medium can be used.

f) Pick human iPSC colonies. On day 21 to day 25 post-transfection, compact human iPSC colonies can be readily visible under microscope. Under microscope, use 20 µl pipet tips to manually pick and transfer each iPSC colony to individual wells of 48-well plates pre-coated with matrix such as Matrigel, Vitronectin or LMN511-E8 in appropriate feeder-free human ESC culture medium.

g) Incubate in a 5 % CO_2 incubator at 36,5 °C ± 0,5 °C and change medium every day.