International Standard

ISO 20012

Biotechnology — Biobanking — Requirements for human natural killer cells derived from pluripotent stem cells

Biotechnologie — Mise en banque de matériel biologique — Exigences relatives aux cellules tueuses naturelles humaines dérivées de cellules souches pluripotentes

First edition
2026-02

Reference number
ISO 20012:2026(en)

© ISO 2026



COPYRIGHT PROTECTED DOCUMENT

© ISO 2026

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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 ... **iv**
Introduction ... **v**
1 Scope ... **1**
2 Normative references ... **1**
3 Terms and definitions ... **1**
4 Abbreviations ... **4**
5 General requirements ... **5**
5.1 General ... 5
5.2 Legal and ethical requirements ... 5
5.3 Personnel, facilities and equipment ... 5
5.4 Reagents, consumables and other supplies ... 5
5.5 Management of information and data ... 6
6 hPSCs and associated data ... **6**
7 Generation of hPSC-NK cells from hPSCs ... **6**
7.1 Processes ... 6
7.2 Unique identification ... 7
7.3 Testing for infectious agents ... 7
7.4 Generation of hPSC-NK cells and culture ... 7
7.5 Subculture and limited expansion ... 7
8 Characterization of hPSC-NK cells ... **7**
8.1 General ... 7
8.2 Viability ... 8
8.3 Morphology ... 8
8.4 Population doubling time (PDT) and subculture/expansion ... 8
8.4.1 PDT ... 8
8.4.2 Expansion ... 9
8.5 Cell population purity ... 9
8.6 Immunophenotyping by flow cytometry ... 9
8.7 Microbiological contamination ... 10
8.8 hPSC-NK cell activity and function ... 10
9 Quality control ... **11**
10 Storage ... **11**
11 Thawing and culturing ... **12**
12 Disposal ... **13**
13 Distribution of hPSC-NK cells — Information for users ... **13**
14 Transport of hPSC-NK cells ... **14**
14.1 General ... 14
14.2 Transport of frozen hPSC-NK cells ... 14
14.3 Transport of living hPSC-NK cells ... 15
Annex A (informative) **Examples of quality control tests for processing of hPSC-NK cells** ... **16**
Annex B (informative) **Examples of methods for the generation of hPSC-NK cells from hPSCs** ... **17**
Annex C (informative) **Example of a quality control test procedure for hPSC-NK cells** ... **19**
Bibliography ... **21**

© ISO 2026 – All rights reserved

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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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.

© ISO 2026 – All rights reserved

Introduction

Natural killer (NK) cells, also known as large granular lymphocytes (LGL), are a type of cytotoxic lymphocytes belonging to the innate immune system. NK cells constitute 5 % to 15 % of the mononuclear cells and up to 20 % of lymphocytic population[1] in the peripheral blood. The expression patterns of activating receptors and inhibitory receptors determine the activating status and functionalities of NK cells. NK cells can eliminate pathogen-infected cells, cancerous cells, and other unhealthy cells by direct cytotoxicity. They express apoptosis-related ligands (TRAIL and FasL) and cytotoxic granules (granzymes and perforin) which can induce cell death in stressed cells. NK cells with the expression of CD16 can also kill antibody-coated target cells by antibody-dependent cell cytotoxicity (ADCC). As innate immune cells, NK cells are also engaged in reciprocal interactions with other immune cells to limit or exacerbate immune responses[2].

In adult, hematopoietic stem cells (HSCs) differentiate into common lymphoid progenitors (CLPs), then, give rise to NK progenitors (NKP). NKPs migrate into all lymphoid tissues or organs, and differentiate into mature NK cells subsequently[3]. However, NK cells can also arise from erythro-myeloid progenitors (EMPs) in the yolk sac. NK cells can be generated by induction from human pluripotent stem cells (hPSCs)[4]. Regarding the various tissue origins of natural NK cells during development, there also has been established various methods for generating induced NK cells from hPSCs. In the NK cell regeneration system, hPSCs are first induced into mesoderm progenitors or lateral plate mesoderm cells via embryo body (EB) formation or monolayer differentiation methods. Then, these cells can be induced into hemogenic endothelial cells (HECs) which further differentiate into hematopoietic progenitor cells (HPCs) via hematopoietic transition (EHT). NK cells can even totally regenerated from these HPCs under specified cytokine combinations[5],[6].

NK cells are fragile and sensitive to cryopreservation and thawing. Standardized approaches to maintain the functionality of banked NK cells is needed. hPSC-dervied NK cell are primary cells, not immortalized, and therefore have a finite life span.

This document is applicable for academic centers, public and private institutions performing NK cell generation from hPSCs (Research and Development) and preclinical studies, not for clinical use.

Importantly, this document is focused on NK cell regeneration that have been reported from hPSCs in culture for research purposes.

EXAMPLE Applications of NK cells (e.g. immunoresponse regulation, anti-tumor or anti-viral)

© ISO 2026 – All rights reserved

Biotechnology — Biobanking — Requirements for human natural killer cells derived from pluripotent stem cells

1 Scope

This document specifies requirements for the biobanking of human natural killer (NK) cells derived from human pluripotent stem cells (hPSCs), including the requirements for the differentiaton, culture, characterization, quality control, storage, thawing and transport of NK cells.

Requirements for the collection of biological source material, the transport to and reception of biological source material and hPSCs at the biobank, as well as the establishment, expansion and QC of hPSCs are covered in ISO 24603.

This document is applicable to all organizations performing biobanking of human NK cells used for research and development in the life sciences.

This document does not apply to human NK cells for the purpose of 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, *Biotechnology — Biobanking — General requirements for biobanks*

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

ISO/TS 23511, *Biotechnology — General requirements and considerations for cell line authentication*

ISO 24603, *Biotechnology — Biobanking — Requirements for human and mouse pluripotent stem cells*

ISO 24190, *Biotechnology — Analytical methods — Risk-based approach for method selection and validation for rapid microbial detection in bioprocesses*

ISO 35001, *Biorisk management for laboratories and other related organisations*

ISO 35001:2019/Amd 1:2024, *Biorisk management for laboratories and other related organisations — Amendment 1: Climate action changes*

ISO 8934-1, *Cell viability analytical methods — Part 1: General requirements and considerations*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 20387, ISO 21709 and the following apply.

© ISO 2026 – All rights reserved

ISO and IEC maintain terminology 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
authenticity
quality of being genuine or true

[SOURCE: ISO/TS 22859:2022, 3.1]

3.2
biorisk
effect of uncertainty expressed by the combination of the consequences of an event (including changes in circumstances) and the associated "likelihood" (as defined in ISO 31073) of occurrence, where biological material is the source of harm

Note 1 to entry: The harm can be the consequence of an unintentional exposure, accidental release or loss, theft, misuse, diversion, unauthorized access or intentional unauthorized release.

[SOURCE: ISO 35001:2019, 3.16]

3.3
cell culture
growth of cells dissociated from the parent tissue by spontaneous migration, mechanical or enzymatic dispersal for propagation under in vitro conditions

[SOURCE: ISO/TS 22859:2022, 3.5]

3.4
cell master file
complete dossier of all procedures and records used to generate cells

[SOURCE: ISO/TS 22859:2022, 3.6]

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 specific markers, genetic polymorphisms and biological activities

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

3.6
cryopreservation
process by which cells are maintained frozen at 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 stem cells into a defined cell state or fate

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

© ISO 2026 – All rights reserved

3.8
flow cytometry
methodologically oriented subdiscipline of analytical cytology that measures cells in suspension in a liquid vehicle as they pass, typically one cell at a time, by a measurement station

Note 1 to entry: The measurement represents transformations of changes in the output of a detector (or detectors) due to changes in scattered light, absorbed light, light emitted (fluorescence) by the cell, or changes in electrical impedance, as the cell passes through the measuring station.

Note 2 to entry: Flow cytometry allows simultaneous evaluation of morphological characteristics of cells (size and internal complexity) with membrane or intracellular antigens.

[SOURCE: ISO/TS 22859:2022, 3.13]

3.9
human NK cells derived from pluripotent stem cells
hPSC-NK cells
<in vitro derived> innate lymphocytes that are differentiated from pluripotent stem cells (ESC and iPSC, CiPSC), which have the abilities of non-specific cytotoxicity, antibody-dependent cell cytotoxicity (ADCC)

Note 1 to entry: Type of immune cell that has granules (small particles) with enzymes that can kill tumor cells or cells infected with a virus. A natural killer cell is a type of white blood cell.

3.10
viable cells
cells within a sample that have an attribute of being alive (e.g. metabolically active, capable of reproduction, possessed of intact cell membrane, or with the capacity to resume these functions) defined based on the intended use

[SOURCE: ISO 20391-1:2018, 3.29]

3.11
cryoprotectant
agents that protect biological material from freezing damage

3.12
vitrification
process that involves rapidly cooling biological material to transform it into a glass-like, amorphous state without the formation of ice crystals, thereby preserving its structure and function

3.13
cell line-derived xenograft
CDX
type of animal model used in cancer research involves implanting human cancer cell lines into immunocompromised mice to study tumor growth and test potential cancer treatments

3.14
antibody-dependent cellular cytotoxicity
ADCC
immune response where antibodies bind to a target cell, marking it for destruction by immune cells such as natural killer (NK) cells

© ISO 2026 – All rights reserved