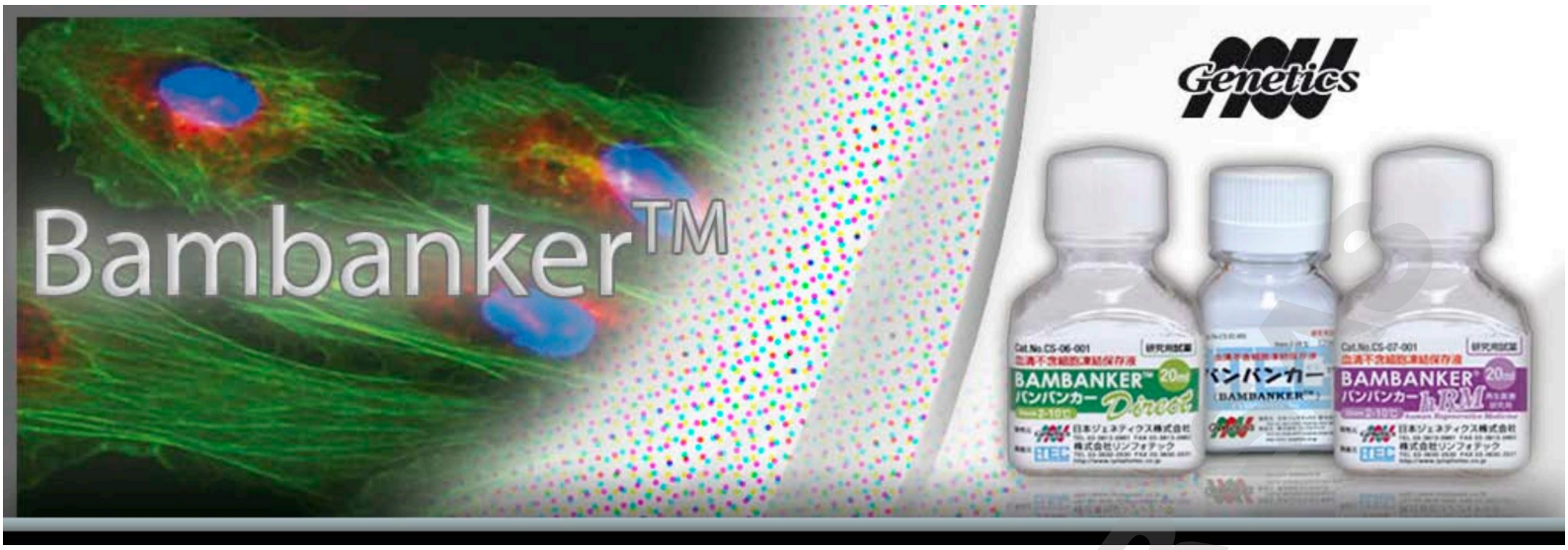

Bambanker™ cell freezing media for cultured cells

It was never easier to freeze and preserve sensitive cell lines

Bambanker™ -
Our Standard Freezing Medium
for sensitive cell lines, like ES and primary cells

NEW!

Bambanker™ - Direct
No centrifugation of cells required
perfectly suited for hybridoma cells and HTP applications

NEW!

Bambanker™ - HRM
made with Human Serum Albumin
no components from animal origin, for use in research only

European patent
EP 1347040

Universal:
All known cell lines can be stored for 12-24 months at -80°C or in liquid nitrogen. The rate of intact cells after thawing is improved significantly compared to traditional media, especially for sensitive cells.

Fast:
Gradual or programmable freezing is no longer necessary. Cells are spun down in the log growth phase and frozen in 1 ml Bambanker™

Serum-free:
No risk of contamination and no interactions with serum proteins and your cells.

Stable:
Bambanker™ is stable if stored at 2-10°C for 2 years.

The freezing medium Bambanker™ offered by the Japanese company Lymphotec was developed initially just for their own R&D projects. They needed a suitable medium for long-term storage of highly sensitive cell lines, such as lymphocytes.
Thanks to the innovative formulation of the new freezing medium, an European Patent (EP 1347040) was granted and the development of this media was made commercially available. Today this innovative cell freezing medium Bambanker™ is the market leader in Japan and characterized by many different published articles with very sensitive cell lines all over the world.

Ordering information

Product	Cat. No.:	Content
Bambanker™ (120 ml)	BB01	120 ml Freezing medium Bambanker™
Bambanker™ (5 x 20 ml)	BB02	5 x 20 ml Freezing medium Bambanker™
Bambanker™ (20 ml)	BB03	20 ml Freezing medium Bambanker™

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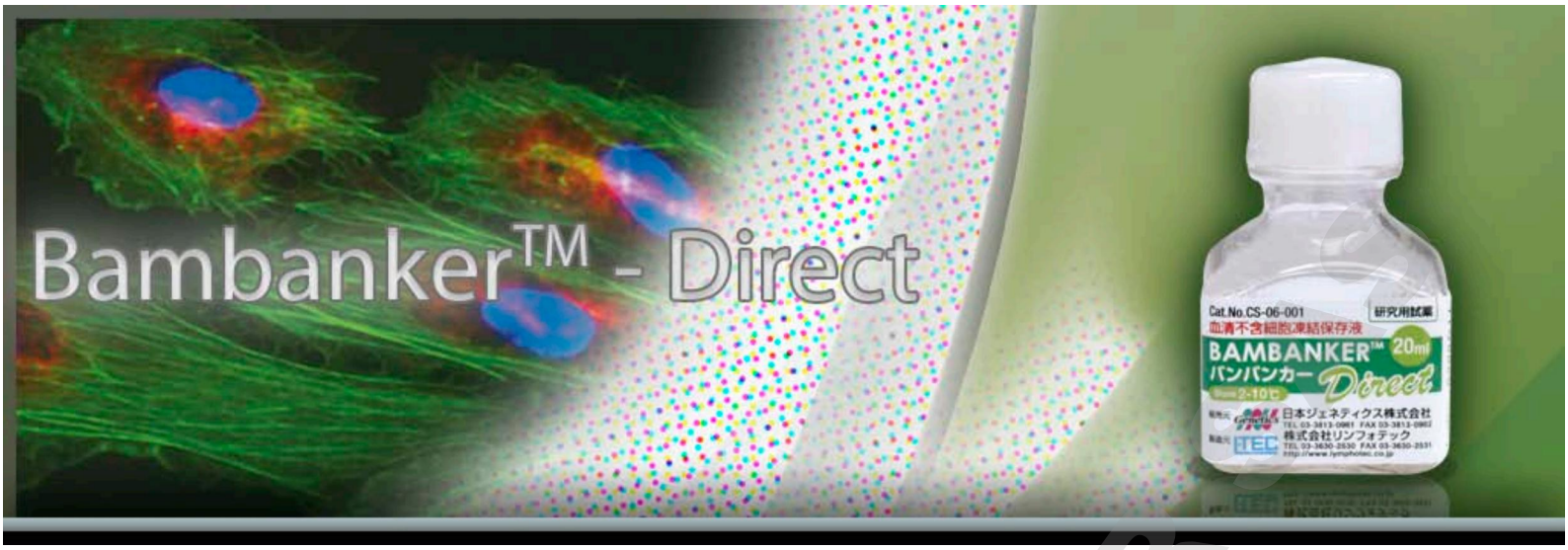
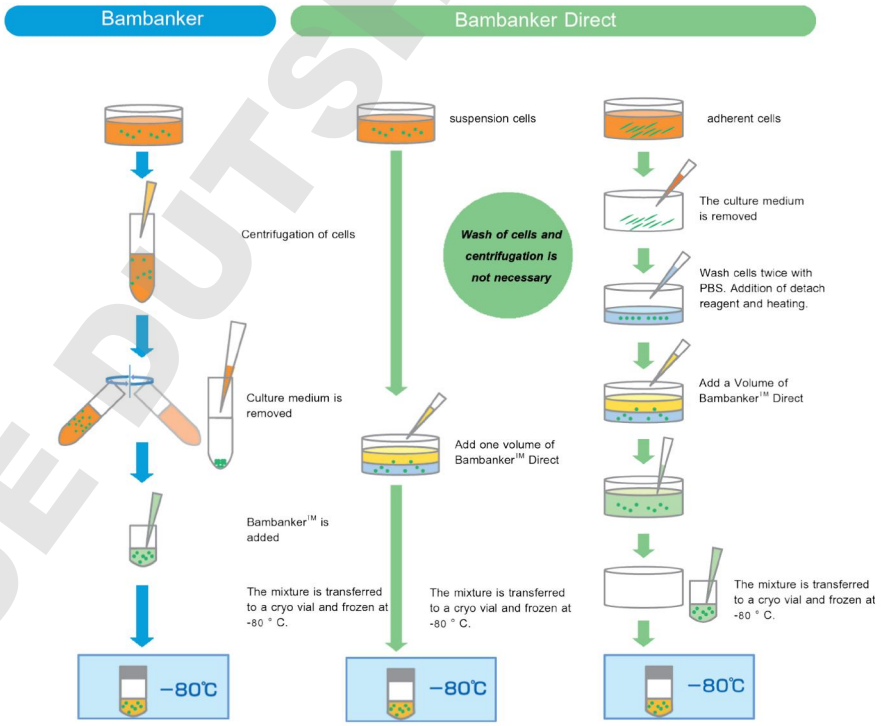


Bambanker™ - Direct

perfectly suited for hybridoma cells and HTP - applications



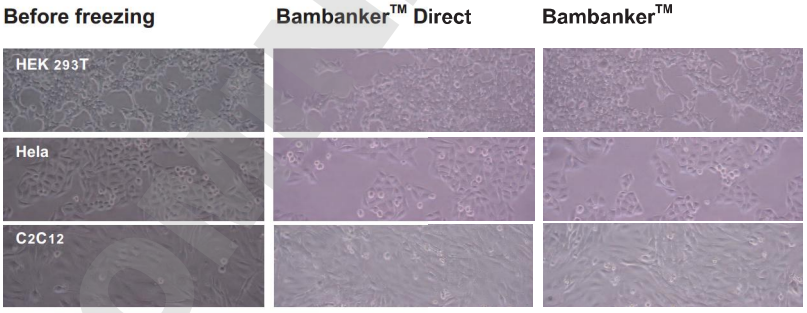


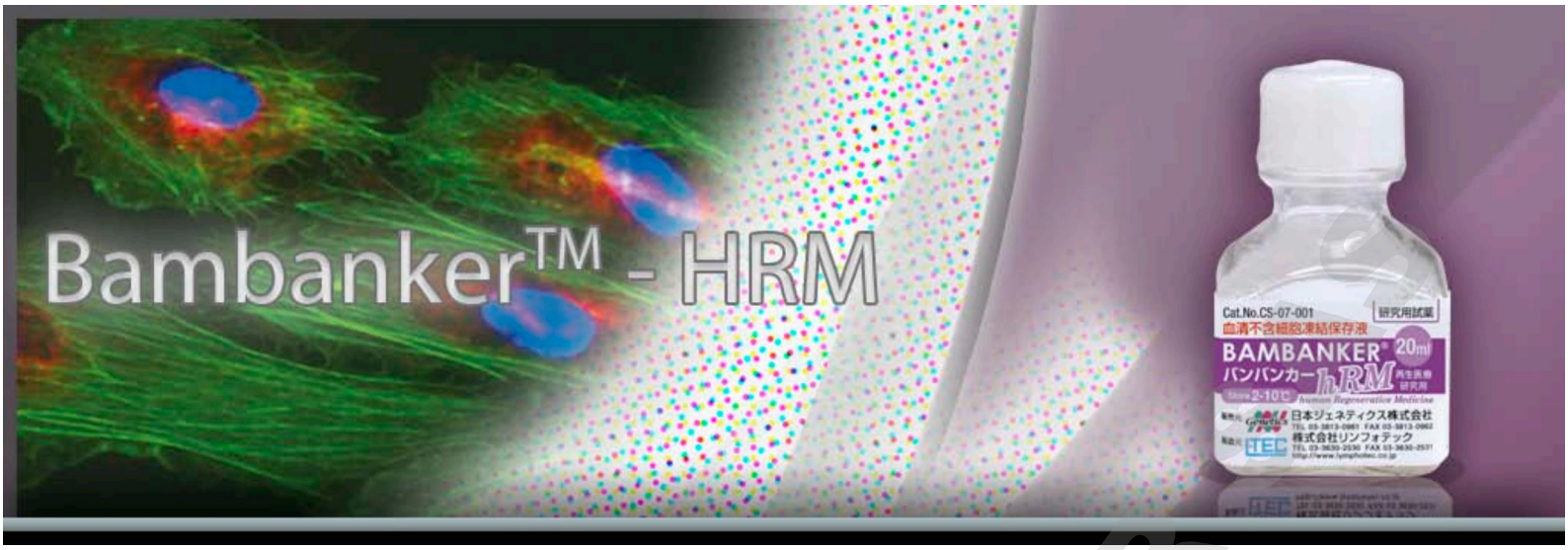
Fig. 2: Three different cell lines before freezing and 12 months after. Cells were stored at -80°C.

Cell lines succesfully stabilized by Bambanker™ Direct:

C2C12 (mouse skeletal muscle cell line); Daudi (human B cell line);
HEK293; HEK293T (human embryonic kidney cell line);
HeLa/HeLa S3 (human cervical cancer cell line); HepG2 (human liver cancer cell line);
Jurkat (human leukemia T cell line); K562 (human chronic myelogenous leukemia cell line);
KATO (human gastric epithelial cell line); MDCK (canine kidney tubular epithelial cell line);
NIH3T3 (mouse embryonic skin cell line) ; OKT4 (mouse hybridoma) ;
OP9 (mouse myeloid stromal cells) ; PC12 (rat adrenal medulla cell line);
SNL (mouse embryonic fibroblasts); ES (mouse embryonic stem cells);
MEF (mouse embryonic fibroblasts); Vero (African green monkey kidney cell line);
P3U1 (mouse myeloma cell line)

Ordering Information:

Product	Cat.No.:	Content
Bambanker™ Direct	BBD01	20 ml Freezing medium Bambanker™ - Direct



Bambanker™ - HRM

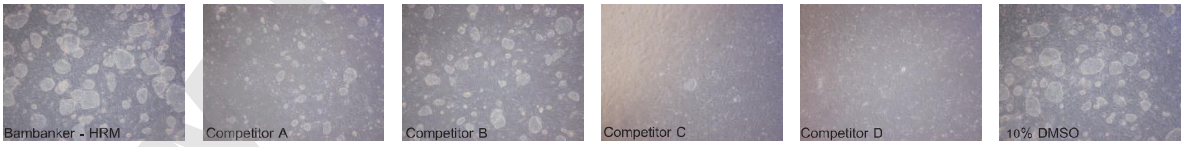
made with Human Serum Albumin, no animal components

Cryopreservation, including freezing and thawing, becomes very important for cell culture technology. Cryopreservation of primate ES and iPS cells is very severe and difficult compared to murine or other cells. At present, vitrification method is considered adequate for primate ES/iPS cell, although slow-freezing method using DMSO has been popular for a wide variety of cell lines. Vitrification method needs special skills and has to avoid dry ice transportation. To address these problems, we developed a new freezing medium (Bambanker™ HRM) containing Human Serum Albumin and DMSO for primate ES/iPS cells.

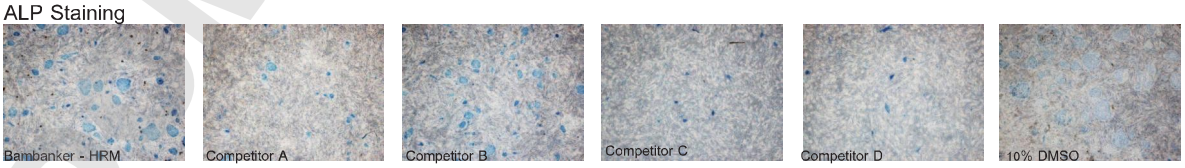
The experimental data shown below describe primate ES cells, cryopreserved in Bambanker™ HRM or 10 %. DMSO/culture medium by slow-freezing or in a conventional vitrification medium by quick-freezing, and then subsequent storage in liquid nitrogen. After 3 days, the cells were thawed by each adequate protocol, and then plated. The different cryopreservation media were analysed by the number of alkaline phosphatase-positive colonies as recovery points. The recovery points of Bambanker™ HRM directly from liquid nitrogen storage were twice higher than that of vitrification medium and four times than that of 10 % DMSO/culture medium. Assuming dry ice transportation, the cryopreserved primate ES cells by above each freezing method were put on dry ice 24 h after three days in liquid nitrogen, and then thawed and plated. The recovery points of Bambanker™ HRM still remained high, but those of vitrification were considerably low.

These results indicate that Bambanker™ HRM provides efficient cryopreservation and dry ice transportation for primate ES/iPS cells. Moreover, as Bambanker™ HRM is a xeno-free and chemically defined, it may be useful for a lot of different applications where hES/iPS cells are involved.

Cells after Freezing and Thawing:



ALP Staining



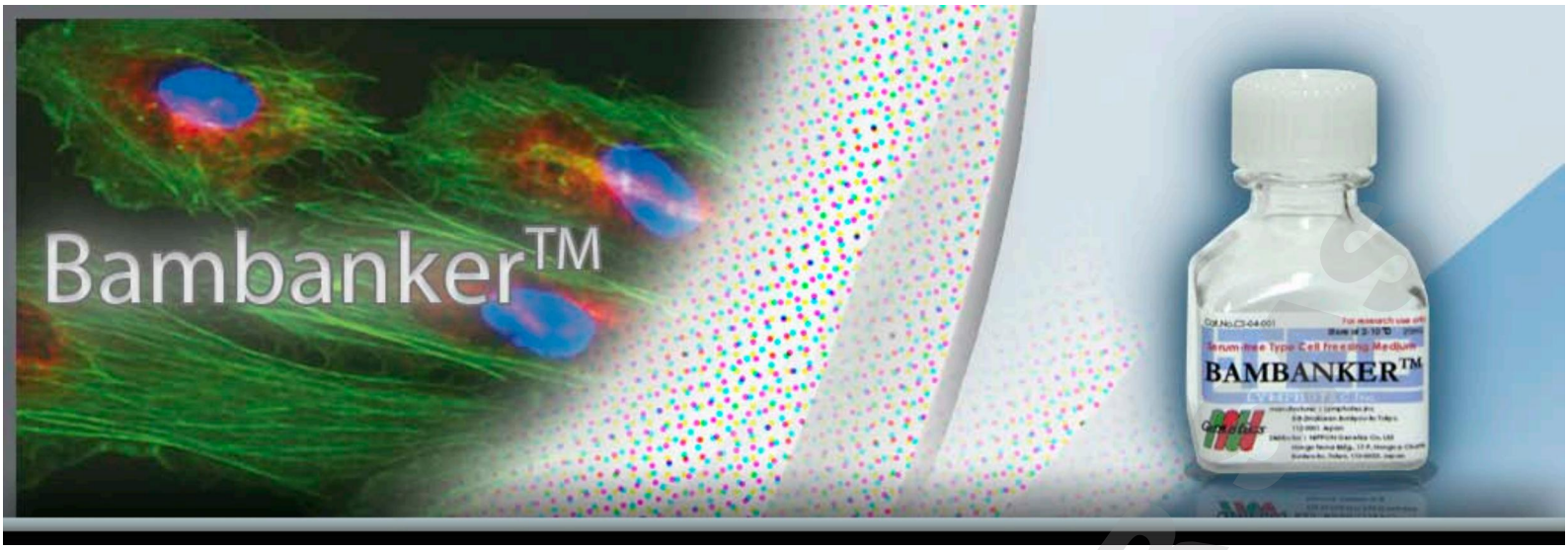
Cell lines succesfully stabilized by BambankerTM HRM:

Human iPS cells;
Neuron cell derived from human iPS cell;
Liver cell derived from human iPS cell;
Heart cell derived from human iPS cell;
Kidney cell derived from human iPS cell;
Human cell lines;
Human primary cell lines;
Human T Cells;
Primate ES cell

Ordering Information:

Product	Cat. No.:	Content
Bambanker™ HRM	BBH01	20 ml Freezing medium Bambanker™ - HRM

Exclusively by Nippon Genetics



Why serum free media?

The qualitative and quantitative composition of serum may be subject to strong fluctuations and each batch can react differently with specific cell types. In addition possible contamination from mycoplasma, viruses, prions or other viral particles can occur. Unidentified ingredients may interact with the cultured cells, which is especially the case if a very sensitive cell line such as embryonic stem cell is used. With the use of Bambanker™ all these barriers are a thing of the past.

Bambanker published papers for:

- embryonic stem cells
- bone marrow stem cells
- dental stem cells
- Osteoblasts
- PBMC
- primary epithelial cells
- embryonic fibroblasts
- lymphocytes
- pig fetal fibroblasts

Stabilization of Mouse Embryonic Stem Cells

Cultivation: 15% FBS/DMEM (1 mM of Sodium pyruvate, 100 µM NEAA, 100 µM of ß-ME, 1000 U/ml of LIF) was used as culture medium. Mouse Embryonic Fibrobasts (MEF) were used as „feeder cells".

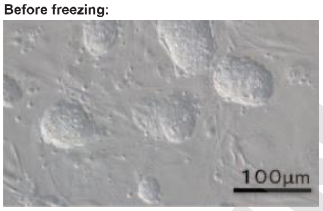
Freezing: Cells were frozen in 5 vials / (60mm dish corresponds to 3.0×10^6 cells/vial). 1 ml/vial of Bambanker™ freezing medium was added and the mixture was directly frozen in -80°C. The following day the vials were transferred to liquid nitrogen (slow freezing).

Thawing: Cells were incubated at 37°C and transferred in cooled culture media. After collection, cells were seeded in 6 well plates and 6 cm dishes.

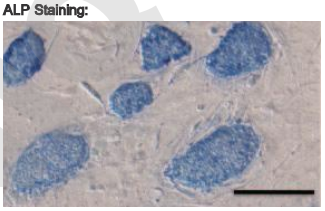
Results: Stabilization of Mouse Embryonic Stem Cells by using Bambanker™ was succesful. Cells were undifferentiated, even after freeze and thaw procedure. No modifications of cells could be observed.

Data were kindly provided by Dr. Ahn (Tokyo Institute of Technology Graduate School of Bioscience and Biotechnology Department of Biomolecular Engineering, Tagawa Laboratory, Japan).

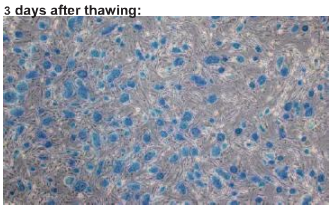
Before freezing:

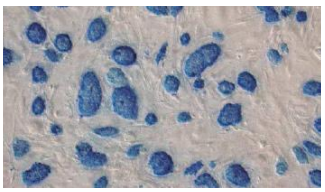


ALP Staining:

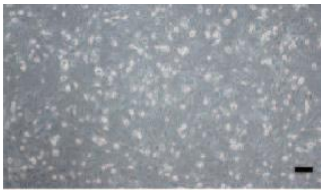


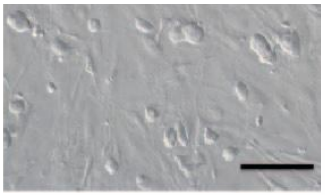
3 days after thawing:





Two days after thawing:





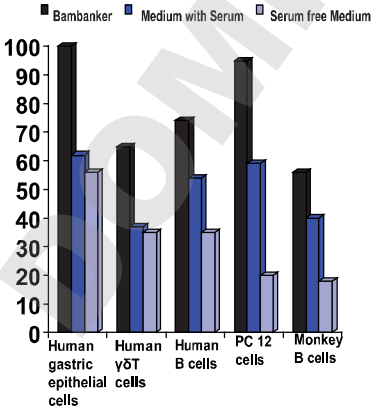


Fig. 2: Rate of intact cells after long term storage. Five different cell lines, preserved in three different media. Cell viability was determined after twelve months.

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