Customers Product Feedback

**Product name**: **Bambanker (BB01)**
Serum-free cryopreservation solution for regenerative medicine research

**Application:** Survival rate after thawing of approximately 1,400 cell types of the cell bank JCRB was low: Improvement of survival rate could be observed for 4 test cell lines stored with Bambanker™

**Cat. No. BB01, BB02**

Data kindly provided by the National Institute of Biomedical Laboratories JCRB cell bank, Dr. Arihiro Ohara.

## Methods

The JCRB cell bank handles approximately 1,400 different cell lines. A low survival rate after thawing frozen cell lines (KHYG-1, KAI3, HL60, OVMANA) has let us to test Bambanker™ and compare it to the up to then used preservation medium for the four cell lines. The growth efficiency after thawing was compared for cells stored with the currently used commercially available preservation medium and Bambanker™. The freezing of the cells were performed with a slow freezing method: The cells were frozen and stored at -80 °C.

Cell preservation method:

| Preservation medium | Freezing method |
|---|---|
| Bambanker™ | Slow method |
| Commercial medium | Slow method |

### Comparison method for three cell lines in suspension :

- KHYG-1 (human NK-like cell line)
- KAI3 (human NK cell line)
- HL60 (human leukemia line)

■ Cryopreservation
- All cultured cells were harvested in the logarithmic growth phase.
- Collection of cells by centrifugation in a centrifugation tube.
- Supernatant discarded after centrifugation.
- Cell precipitate loosen by tapping or light vortexing.
- Addition of 1 ml preservation media to approximately 1 x $10^6$ cells in a storage tube.
- Suspending cells in the preservation medium.
- Storage for 2 weeks at -80 °C.

■ Thawing and cultivation
- Thawing of frozen cells in an 37 °C water bath.
- Suspension of the cells in 10 ml cell culture medium in a centrifuge tube.
- Supernatant discarded after centrifugation.
- Cell precipitate loosen by tapping or light vortexing.
- Resuspension in culture medium. Sample of cells for cell number measurement.
- Incubation at 37 °C and 5 % $CO_2$ in a 96-well plate.
- Daily determination of viable cell number.

## Comparison method for an adherent cell line:

• Adherent cell line OVMANA (human cell line derived from ovarian tumour)

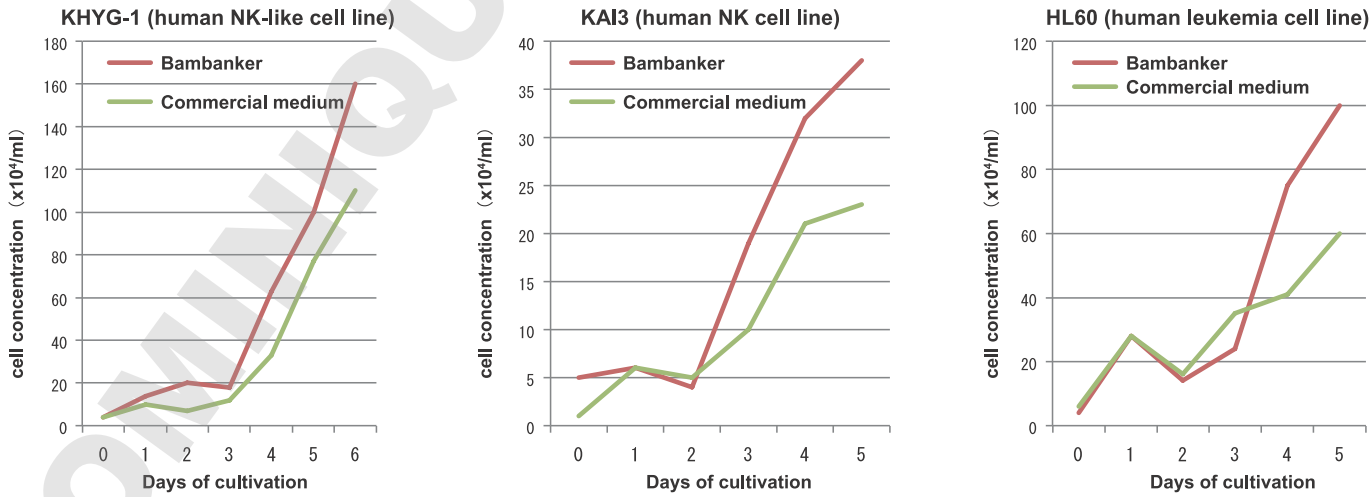
■ Cryopreservation
- Cultured cells were harvested in the logarithmic growth phase.
- Detachment of cells by trypsin treatment.
- Collection of cells by centrifugation in a centrifugation tube.
- Supernatant discarded after centrifugation.
- Addition of 1 ml preservation medium to approximately 1 x $10^5$ cells in a storage tube.
- Mixing of cells with the preservation medium.
- Storage for 2 weeks at -80 °C.

■ Thawing and cultivation
- Thawing of the frozen cells in an 37 °C water bath.
- Suspension of the cells in 10 ml cell culture medium in a centrifuge tube.
- Supernatant discarded after centrifugation.
- Cell precipitate loosen by tapping or light vortexing.
- Resuspension in culture medium. Sample of cells for cell number measurement.
- Incubation at 37 °C and 5 % $CO_2$ in a 96-well plate.
- Detachment of the cells with trypsin treatment after 7 days of culture for cell number determination.

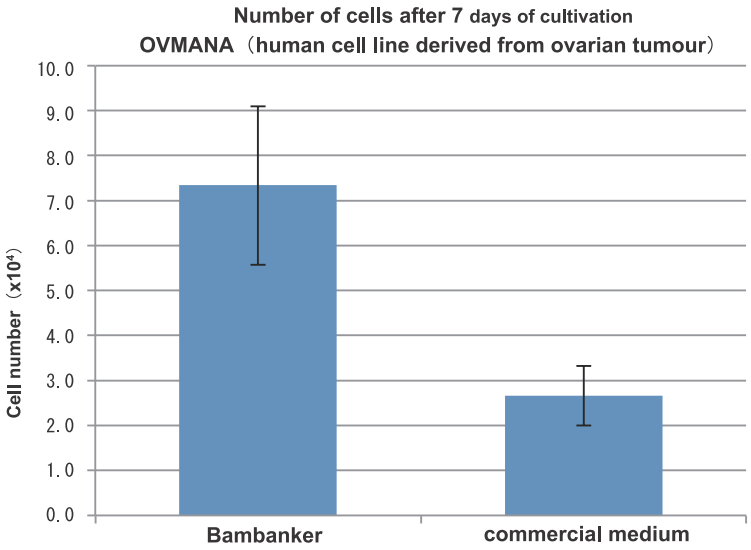
## Results

The results for the above described tests were compared for the both preservation media.

### Cell lines in suspension



## Adherent cell line




| | Bambanker | Commercial product |
|---|---|---|
| Recovery rate immediately after thawing | 32 % | 39 % |

The survival rate after thawing of the four cell lines (KHYG-1, HL60, KAI3, OVMANA) is with the currently used commercially available product and Bambanker™ very low. However after thawing, all four cell lines cell proliferation was improved with Bambanker™ when compared to the currently used commerial product.

<Customers comment>

JCRB cell bank has carried out cell bank business for 30 years and we currently store 1400 types of cell lines. In 2013, we offered about 4,300 ampules for a fee to domestic and foreign researchers. Due to the high number and the wide variety of cell lines, we had some problems. Thus some users complained that their cell lines of dying after thawing, resulting in unsuccessful cultivation. Especially four types of cell lines were a problem which had to be urgently improved. Therefore, we compared Bambanker™ with our currently used commercial preservation medium in a cryopreservation test. The cell lines, which were stored with Bambanker™, showed much higher cell proliferation than cells, which were stored with our currently used commercially available product. Surprisingly, with Bambanker™ we got for all four cell lines very reproducible results.

In addition, Bambanker has no differences between the lots since it does not contain serum. We are very grateful for that because it strongly simplifies the delivery procedure overseas. In the future, we will completly change to Bambanker™ in order to improve the survival rate and growth of our cells.

We are thankful for resolving that long-standing problem and recommend Bambanker™ to all domestic researchers and foreign cell banks.

This data will be also described at our JCRB website. In the near future we plan to test Bambanker™ for human tissue-derived and human iPS cell lines.

NIPPON Genetics EUROPE

**National Institute of Pharmaceutical Laboratories JCRB cell bank** http://cellbank.nibiohn.go.jp/english/

• The JCRB cell bank maintains and prepares cell lines for sale following a strict quality control and characterisation in order to guarantee researchers useful cell lines for their studies. In addition, JCBR has carried out research and development of information and techniques associated with these activities.

• Storage of about 1400 different cell lines.