Reference Note

Freezing spheroidized cells with Bambanker™ hRM

Product	Bambanker™ hRM
Distributor	NIPPON Genetics EUROPE GmbH
Manufacturer	GC LYMPHOTEC Inc.

Reference

This data was provided by the manufacturer, GC LYMPHOTEC Inc.

Summary

Most cryopreservation media are intended to freeze cells in their isolated state. In this reference note, the effect of freezing and thawing on spheroidized cells was tested. The cells were frozen using different cryopreservation media and stored at -80 °C for one week. After thawing, cell state and cell proliferation were examined. The results showed that spheroidized cells frozen in Bambanker™ hRM were unchanged in size and had better cell proliferation, compared to other cryopreservation media. These results indicate that Bambanker™ hRM is a well-suited cryopreservation medium for the freezing of spheroidized cells.

Method

Cell culture:	HEK293T cells	Culture vessel:	96-well plate
Cell medium:	DMEM + 10 % FBS	Conditions:	Incubator 37 °C, 5% CO_2, 90% humidity

Cell freezing and storage:
Cultured HEK293T cells were seeded at 10,000 cells per well to form spheroids. The next day, 10 wells of spheroidized cells were mixed with 1 mL of cryopreservation medium, frozen and stored at -80°C for 1 week.

Evaluation:
Cells were thawed in a water bath at 37 °C and seeded. Cell images were compared immediately after thawing (day 0) and on the third day of culturing (day 3). Also, the absorbance at 450 nM was measured.

Results

Cell images:

The cells were examined under a microscope. The spheroid structure before freezing was compared with the spheroid structure after thawing in different cryopreservation media. The spheroid size was larger after thawing with Bambanker™ hRM and Product A than after thawing with Product B. The size difference was even more evident after three days.

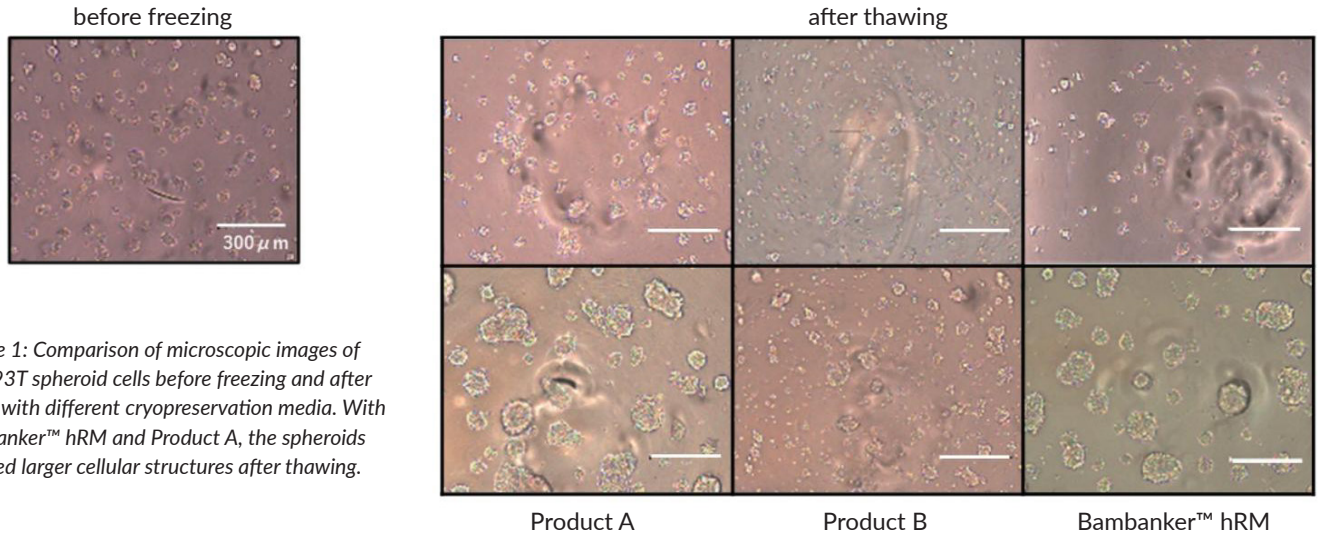

Figure 1: Comparison of microscopic images of HEK293T spheroid cells before freezing and after thawing with different cryopreservation media. With Bambanker™ hRM and Product A, the spheroids showed larger cellular structures after thawing.

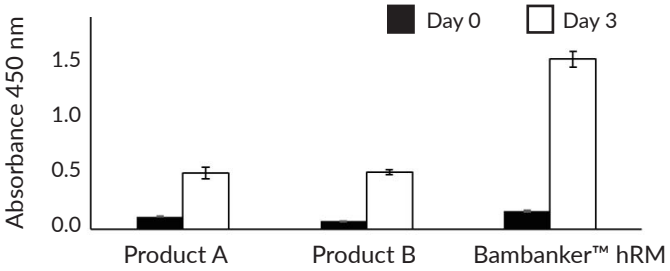

Figure 2: Cell proliferation was determined by measuring the absorbance at 450 nm. Compared to Product A and Product B, Bambanker™ hRM showed the highest proliferation value after three days of cultivation.

Cell proliferation:

Cell proliferation was measured by absorbance at 450 nm on day 0 and after three days of cultivation (day 3). The difference in cell proliferation after freezing the spheroidized cells in different media was particularly evident on day 3. Spheroidized cells preserved with Bambanker™ hRM showed the highest absorption value at 450 nm.

Conclusion

The structure and size of the spheroids after thawing with Bambanker™ hRM were comparable to the spheroid structure and size before freezing. Also, Bambaker™ hRM showed the highest value of cell proliferation after spheroid thawing. These results indicate that Bambanker™ hRM can be used very effectively for freezing and storage of spheroidized cells at -80 °C and performs better than other cryopreservation media.

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