2014.06

Rev. 2022.01

Application consideration

Please keep the following points in mind:

RCB1296 : HNT-34　Lot.4

The cells can be easily damaged by thawing process.
Please thaw **only one vial at a time**, and the thawing process should be completed **as quickly as possible**.
After thawing process, please immediately transfer the cells into an incubator.

The cells should be centrifuged **at room temperature**.
Centrifuge the cells **at 1,000 rpm once**, and suspend the cells by **gentle pipetting**.

This lot contains 5.3 x 10^6 cells/tube.
Maintaining high cell density is important. In particular, cell density just after thawing process is very critical, and it will largely affect the subsequent cell growth.
In the case of using 35mm culture dishes, please suspend the cells in 4 mL culture medium and seed into 2 dishes (2 mL/dish).
When using other culture vessels, seed the cells at a concentration of about 1x10^6 cells/ml.

Next day after thawing, cell viability will be decreased to 40% and dead cells will start to aggregate and float. However, the condition will recover in a few days.* **Therefore, please observe the cells without any operation (e.g., collecting and centrifugation, medium change, medium addition and so on)**.
Please wait till the cells start to proliferate like C (photo #5 and #6) in the backside of this page, and then passage the cells at 1:2 ~ 1:4 dilution.
*Days required for recovery will vary depending on thawing process (about 3 ~ 7 days).

If the cells start to proliferate vigorously, they can be passaged at 1:4 ~ 1:8 dilution.
In turn, when maintaining the cells, **please pay attention to avoid overgrowth (i.e., excessively high concentration of cells)**.
Photos (thawing and passaging) are on the backside of this page.

【Technical support】e-mail : cellqa.brc@riken.jp

A Just after thawing: Most of cells have well-defined borders and round shape.

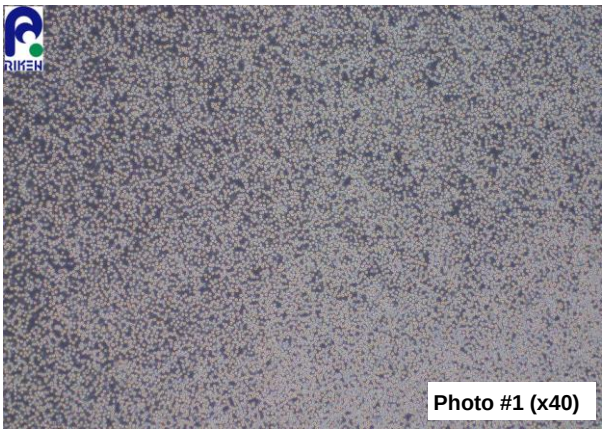
Photo #1 (x40)

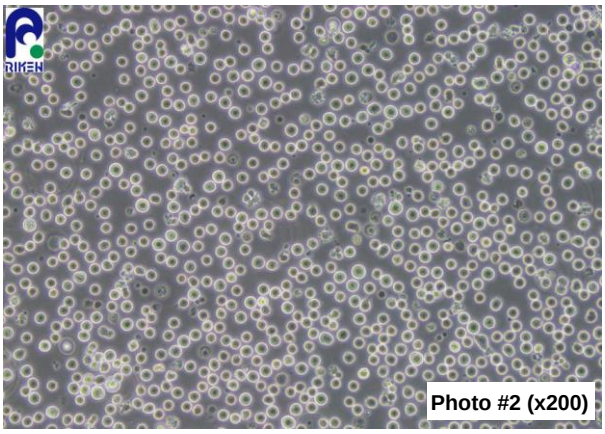
Photo #2 (x200)

B Next day after thawing: Cell viability is decreased to 40% and dead cells start to aggregate and float.

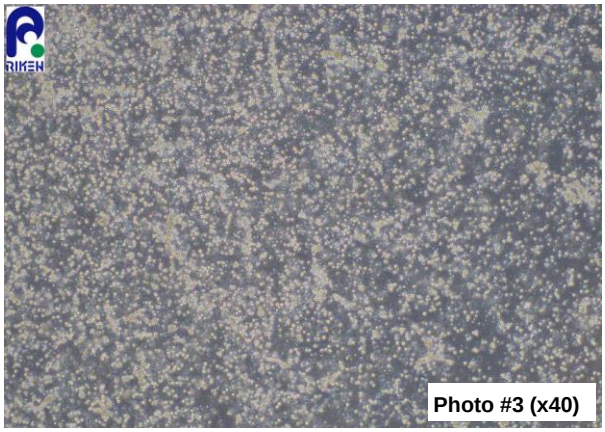
Photo #3 (x40)

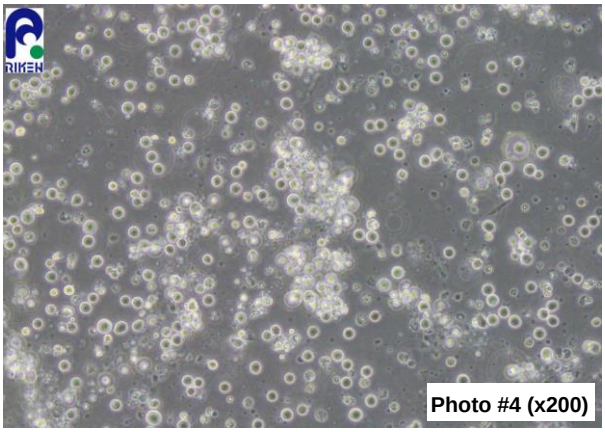
Photo #4 (x200)

C Day 5 after thawing: Actively proliferating cells are observed and the cells are recovered. → 1st subculture at this point.

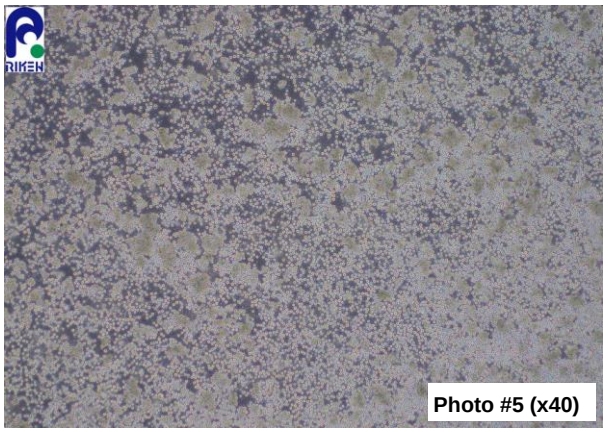
Photo #5 (x40)

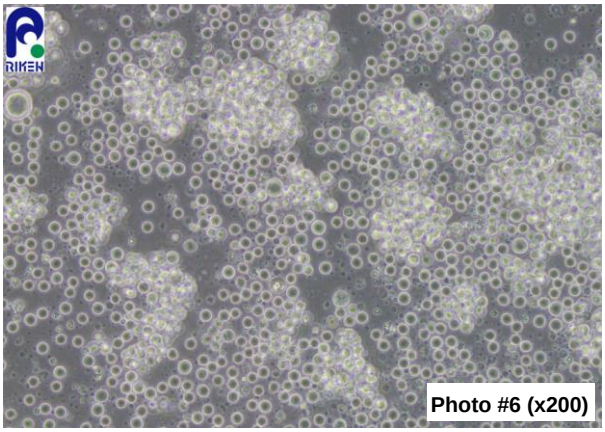
Photo #6 (x200)

D Next day after subculture: The cells above (C) were diluted in 1:4 with fresh medium.

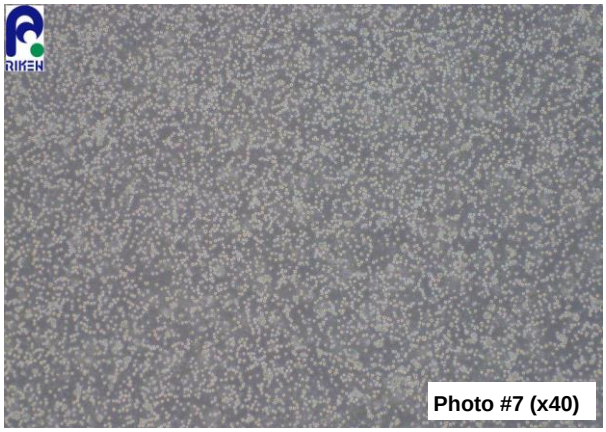
Photo #7 (x40)

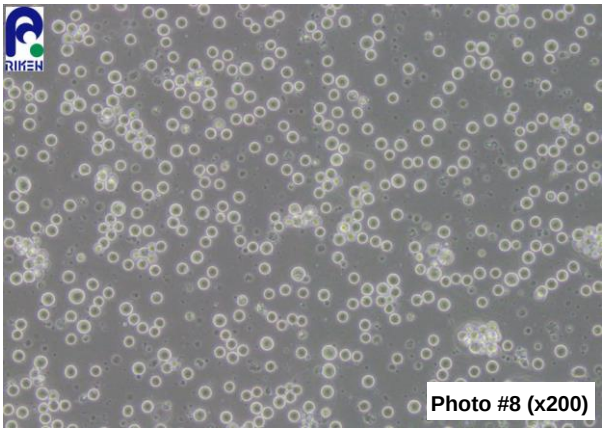
Photo #8 (x200)

*Caution: During the time period from A to C, do not perform any culturing operation (e.g., collecting and centrifugation, medium change, medium addition and so on)