2010-04

Rev. 2017-03

# Application consideration RCB0558 : LLC

Please keep in mind following points:

・NOTE: Regarding this cell line, it is very important to control cell density.

・Please start cultivating cells with either two 60mm dishes or two T-25 flasks per tube (or per ampoule).

・If you start cultivating cells after counting cell number, please plate cells with either $5x10^5$ cells per 60mm dish or T-25 flask.

・**Please make sure to observe the condition of cells on the next day of starting cultivation.**

Regarding the condition of cells during culture.

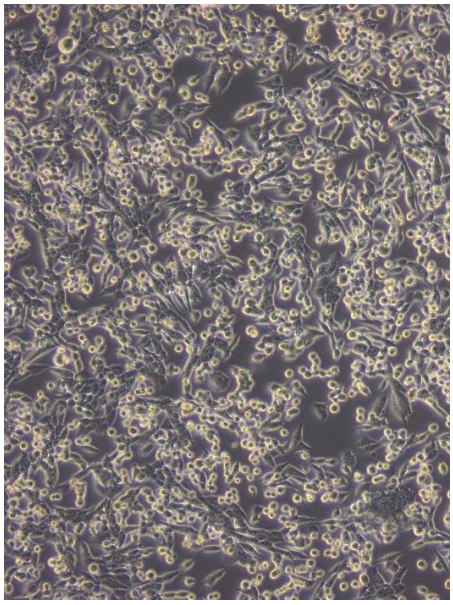
(A) refers to the best condition of cells. When cells overgrow, they start becoming round and detaching from dishes or flasks. When cells reach 70-80% of the bottom surface of dishes or flasks, please subculture them with 4-8 dilutions.

(B) refers to the condition of overgrowth. Since some cells are aggregated, they are easily detached. Therefore, please subculture them right away in order to recover the original condition.

(C) refers to the condition of low viability due to lowering cell density too much.
Please be careful not to make this condition, otherwise you will not be able to recover cell viability anymore.

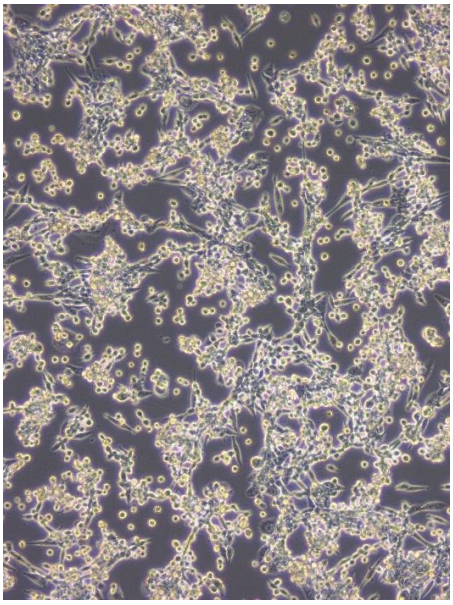
・Freezing cells:

Please freeze cells under the good condition as (A) by observing them as carefully as when you subculture them, and note that cells that proliferate drastically (in the logarithmic phase) show better preservation efficiency.



(A) Good condition

Cells are attached in proper density.

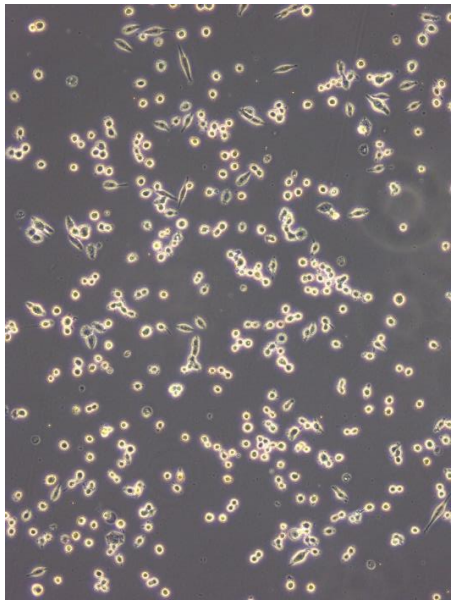


(B) Poor condition

〈Cell overgrowth〉

Cells became rounded shape and turned inward.

(In this condition, even though cell density is less than 70%, cells might possibly start detaching from dishes. Therefore, it is recommended to subculture cells as soon as possible.)



(C) Bad condition

〈Cell density is too low〉

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