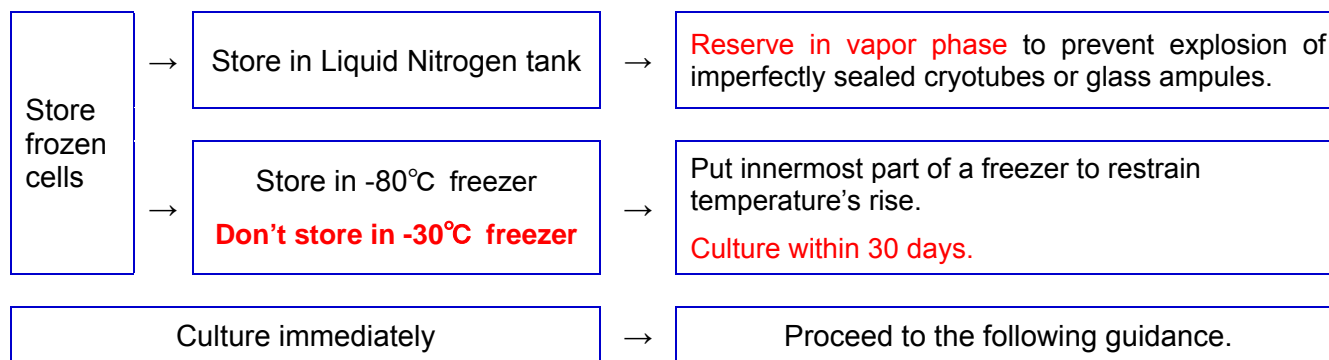


## After you receive cell lines

Transfer quickly from the package to storing place or culturing.

Frozen cells in above  $-65^{\circ}\text{C}$  will be rapidly damaged.



## First Culturing Guidance

### Media and equipment

- Use a  $25\text{cm}^2$  flask or a 6cm dish (or smaller equipment if it is specified in data sheet) to avoid excessive dilution.
- Prepare the appropriate media specified in the data sheet of the web catalog.

If you have any question, please inquire with JCRB Cell Bank **before you start culturing**.

【Q&A: the reference page in JCRB web site】

[http://cellbank.nibiohn.go.jp/english/other\\_e/other\\_faq\\_e.html](http://cellbank.nibiohn.go.jp/english/other_e/other_faq_e.html)

### Cell lines should be thawed rapidly

【Handle one by one. Don't thaw two or more ampules together.】

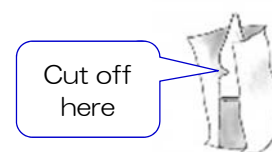
1. Take out one ampule from the package. (Use safety gloves and a face shield)
2. Immediately put the ampule in warm (not higher than  $37^{\circ}\text{C}$ ) water, and thaw the content within 2 min by shaking.



### Seed at $25\text{cm}^2$ flask or 6 cm dish

【Under aseptic conditions】

3. Prepare 10 ml of the specified medium in a centrifuge tube.
4. Sterilize the ampule by permeating gauze with 70% ethanol or cationic detergent sterilizer
5. Wrap the ampule with sterilized gauze, and snap off the neck of the ampule with care.
6. Transfer the cell suspension to a centrifuge tube.
7. Centrifuge the mixture at 1000 rpm for 5 min, and discard the supernatant.
8. Without washing, re-suspend the cells in 5ml of the same medium.



*If especially the media volume is recommended in the data sheet, follow the instruction.*

9. Culture them in a  $25\text{cm}^2$  flask or a 6 cm dish for tissue cultures.

*The cell density given in the attached data sheet is standard density for logarithmic growth phase. It's not the actual density on thawing.*

10. Make sure of cell proliferation before proceeding to passage. *It may be needed for some days or a week for proliferation.* After growing well, make frozen stocks early.