NIBN
JCRB Cell Bank

After you receive cell lines

Transfer quickly from the package to storing place or culturing.
Frozen cells in above -65℃ will be rapidly damaged.

Store frozen cells	→	Store in Liquid Nitrogen tank	→ Reserve in vapor phase to prevent explosion of imperfectly sealed cryotubes or glass ampules.
	→	Store in -80℃ freezer **Don't store in -30℃ freezer**	→ Put innermost part of a freezer to restrain temperature's rise. Culture within 30 days.
Culture immediately			→ Proceed to the following guidance.

First Culturing Guidance

Medium and equipment

- ○ Use a 25cm^2 flask or a 60mm dish (or smaller equipment if it is specified in data sheet) and 5ml (or smaller) medium to avoid excessive dilution.
- ○ Prepare the appropriate medium 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

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℃) water, and thaw the content within 2 min by shaking.

Seed at 25 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 cm^2 flask or a 60mm 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.