How to Start a Culture of a JCRB-supplied Frozen Cell Line

National Institutes of Biomedical Innovation, Health and Nutrition JCRB Cell Bank

After you receive cell lines

Do not place in at –30 °C.

Cell lines should be stored in liquid nitrogen vapor or cultured immediately after arrival. If the cell lines would be stored in a freezer, they should be kept below –80 °C, and should not be stored longer than 30 days. Do not store at –30 °C. It will result in rapid loss of viability.

Do not immerse in liquid nitrogen directly!!!

If cryotube or glass ampoule has been stored directly in liquid nitrogen (liquid phase), there is a risk of exploding imperfectly sealed cryotube or glass. It is recommended to preserve them in vapor phase of liquid nitrogen tank.

Thawing

Cell lines should be thawed rapidly.

Take out the ampoule from the styrene foam package and thaw the content within 2 min by shaking in warm (not higher than 37°C.) water. Use safety gloves and a face shield to avoid injury from possible explosion of the ampoule. It is safe to thaw them one by one, even if it takes longer.

Seed at 25 cm2 flask or 6 cm dish.

All operations should be performed under aseptic conditions. Sterilize the entire surface of the ampoule with gauze moist with 70% ethanol or cationic detergent sterilizer, and cut the neck off while wrapped in sterilized gauze. Although every ampoule has a groove around the neck for easy cutting off, a precaution may be required to open the ampoule because of its hard structure. Transfer the cell suspension to a centrifuge tube, add 10 ml of the medium specified in the catalog, centrifuge the mixture at 1000 rpm for 3 min, and discard the supernatant. Without washing, resuspend the cells in the same medium and culture them by the standard procedure. Unless otherwise specified, the appropriate culture volume would be 5 ml per ampoule in a 25 cm2 flask or 6 cm dish for tissue cultures. The cell density given in the attached document or in the catalog may differ from the actual density on thawing. Make sure of cell proliferation before proceeding to passage, because excessive dilution may lead to cell death particularly in hemocytes and lymphocytes.