

## Protocol

# Cryopreservation protocol using PentaHibe<sup>®</sup> Complete

PentaHibe<sup>®</sup> Complete is a ready-to-use formulation developed for cryopreservation of primary cells. The product fits into (your) standard cryopreservation work-flow.

### Materials and reagents:

- 100% PentaHibe<sup>®</sup> Complete
- Ice

## 1 Cryopreservation procedure

### Protocol (example)

1. Pellet your cells at a centrifugation speed adjusted to the specific cell type
2. Carefully discard the supernatant and gently resuspend cell pellet in cold (2-8°C) PentaHibe<sup>®</sup> Complete solution
3. Incubate on ice for 5 min
4. Freeze cells using either a controlled rate freezer (e.g. start temp 4°C, -1°C/min drop to 0°C, -2°C/min drop to -45°C, and -5°C/min drop to -100 °C), followed by storing cells in a liquid nitrogen container, or place the vials in an appropriate freezing container with a -1°C/min drop to -80°C followed by transferring vials to a nitrogen container

## 2 Thawing

1. Thaw cells rapidly in a 37°C water bath. Thawing should be done gently by swirling the sample until all visible ice has just melted
2. Immediately dilute the mixture of thawed cells with appropriate culture medium pre-warmed to a temperature of 20-37°C at a dilution ratio of 1:10 (sample to culture medium)
3. Centrifuge and remove the supernatant and resuspend cells in appropriate culture medium. Cells are ready to be processed