PentaHibe®

PHARMACOSMOS

Protocol

DMSO-reduced cryopreservation using 10% Pentahibe® Base, 2% DMSO and 4% human albumin

The specific protocol for freezing cells depends on the cell type and cryopreservation solution used. For T cells and Mesenchymal Stem Cells, below protocol has proven successful[1,2].

Materials and reagents:

- 40% Pentahibe® Base
- 20% human albumin (HA)
- 5 % human albumin (HA)*
- 99% DMSO
- Distilled water
- Ice

* Please use a preformulated 5% human albumin solution. Do not use a 20% solution diluted with water

1 Preparation of cryopreservation solution

Formulation

On the day of use prepare a cryopreservation solution containing 10% Pentahibe® Base, 2% DMSO, 4% HA, e.g 50 mL 1x concentration by:

1. Transfer 12.5 mL of Pentahibe® Base to a tube
2. Add 3 mL of 20% HA and mix the solution gently
3. Add 27.5 mL of 5% HA
4. Add 1 mL of 99.9% DMSO
5. Add 6 mL of distilled water and mix the solution gently
6. Keep the cryopreservation solution on ice until use

2 Cryopreservation procedure

Protocol (example)

1. Pellet your cells at a centrifugation speed adjusted to the specific cell type
2. Discard the supernatant and gently resuspend cell pellet to the desired cell concentration with the cryopreservation solution. Aliquot 1 mL into 1.8 mL cryovials
3. Incubate on ice for 5 min
4. Cells are frozen using a controlled rate freezer e.g. utilizing gradient-based cooling (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

3 Thawning

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

[1] Haastrup EK et al. DMSO (Me2SO) concentrations of 1-2% in combination with pentaisomaltose are effective for cryopreservation of T cells. Transfusion and Apheresis Science 2021, Aug:60(4):103138.

[2] Svalgaard JD et al. Cryopreservation of adipose-derived stromal/stem cells using 1-2% Me2SO (DMSO) in combination with pentaisomaltose: An effective and less toxic alternative to comparable freezing media. Cryobiology 2020, Vol. 96(01):207-213.

We are here to answer your questions

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