

## **PROCEDURE FOR MAKING FREEZE SOLUTION**

Cells require the correct amount of DMSO and nutrient for sustainability when cryopreserved and thawed. **STERILE TECHNIQUE SHALL BE USED FOR PREPARATION OF THIS SOLUTION**

1. Determine the correct amount of freeze solution required for the cryopreservation of the cells in question. For this process there needs to be an equal volume of freeze solution to cell suspension. Ideal cell freezing conditions are where the cell concentration is between 5-50 million cells per milliliter of total volume. When cells are either at a lower or higher cell concentration there can be cell death.
2. Our current freeze solution is composed of 3 ingredients at certain concentrations. They are, 40% Normosol-R (an injectable saline solution), 40% Human Serum Albumin (25% by volume), and 20% DMSO (dimethyl sulfoxide). When these ingredients are mixed, and this mixture is added to an equal volume of cell suspension the DMSO concentration will drop to 10%. That is the ideal concentration for cryopreserving lymphocytes. It is best to make 1-3 ml more freeze solution than what is required. (AN EXAMPLE) If you need 10 ml freeze solution, it is best to make 12 ml of freeze solution. So, you will need to combine 4.8 ml Normosol-R, 4.8 ml Human Serum Albumin, and 2.4 ml DMSO. Then, 10 ml freeze solution will be added to 10 ml of cell suspension.
3. Obtain a 50 ml Falcon tube. Add the determined amount of chilled Normosol-R.
4. Then add the determined amount of chilled Human Serum Albumin to the Normosol-R in the Falcon tube.
5. Lastly, add the determined amount of DMSO to the mixture of Normosol-R and Human Serum Albumin in the Falcon tube. The DMSO will need to be added slowly, dropwise to the mixture. While adding the DMSO carefully shake or swirl the Falcon tube to distribute the DMSO into the mixture. Adding DMSO causes an exothermic reaction and the heat will need to be dissipated. Using chilled Normosol-R and Human Serum Albumin will temper the buildup of heat from the reaction.
6. Once all the DMSO has been added chill the mixture in the refrigerator for 10 minutes or longer. Larger volumes require more time to chill.
7. Once chilled the freeze solution is ready for use. This solution will be added in equal volumes to the cell suspension that needs cryopreservation.

## **MATERIALS NEEDED FOR THIS PROCEDURE**

- Normosol-R
- Human Serum Albumin (25% by volume)
- DMSO pharmaceutical grade
- Syringes
- Needles
- Falcon tubes