Instruction for Use

PSI Pentastarch Solution in Bags
Product Code: PST001

DIRECTIONS FOR PREPARATION AND USE

DESCRIPTION
PSI Pentastarch Solution is composed of the following:

Pentastarch	20% (w/v)	200	g/L
Dextrose	0.18% (w/v)	1.8	g/L
Electrolyte Solution†		q.s. to volume	

†Electrolyte Solution (per 100 mL volume) is composed of :
526mg NaCl, 502mg Sodium Gluconate, 368mg Sodium Acetate·3H_2O, 37mg KCl, 30mg $MgCl_2$·6H_2O

PSI Pentastarch Solution is a clear, sterile liquid product intended for use as a component in freezing cells. Osmolality of undiluted solution is approximately equal to 360 mOsm/kg; pH is approximately 6.6, but may require verification and adjustment according to user specification at the time of use.

HOW SUPPLIED – STORAGE CONDITIONS
50 mL PSI Pentastarch Solution is contained in a 100 mL capacity, flexible EVA bag. A shelf carton contains 5 bags. Store PSI Pentastarch Solution at 2-8°C (36-46°F). Avoid excessive heat. Do not use if obvious particulate matter, precipitates, or contamination are evident in the solution.

ACTIONS
Pentastarch acts as an extracellular, protecting agent and when mixed with intracellular cryo-preservatives, will aid in the preservation of cells during the freeze/thawing process.

INTENDED USE
PSI Pentastarch Solution is a liquid product intended for use as a component in cryopreservation media for human *ex vivo* cells, as part of cell culture processing.

PRECAUTIONS
PSI Pentastarch Solution is not intended to be directly added to cells. PSI Pentastarch Solution is normally added to cells at about a 12% starch concentration, having been mixed with DMSO and serum/albumin (see Method of Utilization, below). Failure to add other components in order to obtain a final cryopreservation media may have deleterious effects on cells.

Components should not be mixed and held in the original bag containing PSI Pentastarch Solution. DMSO may dissolve plastic and thereby contaminate the final cryopreservation media. Furthermore, cells should not be frozen directly in the initial bag.

During the use of PSI Pentastarch Solution, follow the details of your institution's instructions for the processing of Human Cells, Tissues, and Cellular Tissue-Based Products (HCT/Ps), in compliance with the applicable Current Good Tissue Practice regulations, during the use of PSI Pentastarch Solution.

METHOD OF UTILIZATION

Preparation of Cryopreservation Media from PSI Pentastarch Solution

Cryopreservation media, often called 'freeze mix', may be prepared from PSI Pentastarch Solution by the addition of both a cell permeant and a serum/albumin fraction in order to maintain cell function and viability during and after freezing. A typical method for the preparation of 52 mL of cryopreservation media is:

1. Aseptically add 16 mL of 25% human serum albumin (HSA) to 31 mL of PSI Pentastarch Solution.
2. Aseptically add 5 mL of dimethyl sulfoxide (DMSO) to the mixture of HSA and Pentastarch solution.
3. Final concentration of the cryopreservation media (freeze mix) is 8% HSA, 12% Pentastarch, 10% DMSO. Freshly prepared freeze mix may be stored for up to 24 hours, at 2-8°C.

Use of Freeze Mix (Cryopreservation Media) for Freezing Cells

A typical method for the use of fresh (≤ 24 hours storage time at 2-8°C) freeze mix is as follows:

1. Obtain a clean cell suspension in the preferred media (i.e. any trypsin or other unwanted substances are washed/deactivated). Optimal concentration of cells is on the order of 10^6 cells/ml.
2. Add an equal volume of freeze mix to a volume of cell suspension (1:1 mixing ratio). This yields final concentrations of 4% serum/albumin, 6% Pentastarch, and 5% DMSO. Mix well.
3. Transfer 1mL aliquots of freeze mix + cell suspension mixture into sterile, cryogenic vials.
4. Place aliquots into a controlled-rate freezer, a foam cooling device, or similar apparatus, to ensure a constant rate of cooling of -1°C/minute.
5. Store frozen aliquots in a freezer at -80°C (weeks to months), or in vapor phase liquid nitrogen (months to years) according to institutional guidelines.

The above protocols are taken in part from methods developed at NIH Clinical Center and inspired by the following:

REFERENCES

Stiff PJ, Koester AR, Weidner MK, Dvorak K, and Fisher RI. Autologous bone marrow transplantation using unfractionated cells cryopreserved in dimethylsulfoxide and hydroxyethyl starch without controlled-rate freezing. *Blood* 70(4): 974-978, 1987.

Rowley SD, Feng Z, Chen L, Holmberg L, Heimfeld S, MacLeod B, and Bensinger WI. A randomized phase III clinical trial of autologous blood stem cell transplantation comparing cryopreservation using dimethylsulfoxide vs. dimethylsulfoxide with hydroxyethylstarch. *Bone Marrow Transplantation* 31: 1043-1051, 2003.

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