CryoSure-DEX40

Cryoprotectant for the cryopreservation of hematopoietic stem cells to protect cells from freezing injuries during freezing and thawing

Kryoprotektivum zur Kryokonservierung hämatopoetischer Stammzellen zum Schutz vor Gefrierschäden während des Einfrierens und Auftauens

Sterile acc.to EP/USP
Steril gem. EP/USP

Pyrogen-free acc. to EP
pyrogenfrei gem. EP

Endotoxin-free acc. to EP/USP
endotoxinfrei gem. EP/USP

Free of Mycoplasma acc. to EP
Frei von Mykoplasmen gem. EP

Contains: 55 g/dl DMSO USP Grade, 5 g/dl Dextran 40 USP Grade, Water for injection EP
Inhaltsstoffe: 55 g/dl DMSO USP Grade, 5 g/dl Dextran 40 USP grade, Wasser für Injektionszwecke EP

WARNING: Do not autoclave.
ACHTUNG: Nicht autoklavieren.

Do not use unless solution is clear.
Nur klare Lösung verwenden.

Not for Injection.
Nicht injizieren.

STERILE A

Sterilized by sterile-filtration
Steril durch Anwendung aseptischer Verfahrenstechniken

Store at 2°C – 8°C.
Bei 2°C – 8°C lagern.

Protect from strong light.
Vor Sonnenlicht geschützt aufbewahren.

LOT
Lotnumber: see product labelling
Chargennummer: siehe Produktetikett

Use until: see product labelling
Verwendbar bis: siehe Produktetikett

REF
WAK-DEX40-25 (25 x 8 ml)
Instructions for use CryoSure-DEX40

Introduction:
CryoSure-DEX40 is a ready-to-use cryoprotective solution for the addition to a volume reduced buffy coat suspension from cord blood according to the method of Rubinstein et al (1). CryoSure-DEX40 is a solution consisting of 50 % v/v DMSO and 50 % v/v of a 10% aqueous solution of Dextran 40.

DMSO (Dimethyl Sulfoxide) is a cryoprotectant which penetrates the cell wall and takes its cryoprotectant effect within the cell. It reduces the osmotic stress on the cells during freezing and thawing (2, 3, 4, 5, 6) and antagonizes the osmotic shock (7). Also DMSO protects the cells by reducing dehydration and shrinkage of the cells during the freezing process (5, 8). After thawing DMSO has to be removed from the stem cell suspension by means of wash centrifugation.

According to the protocol of Rubinstein et al the DMSO-concentration in the volume-reduced ready-to-freeze endvolume is 10% v/v. Before freezing and after thawing DMSO is potentially cytotoxic. The cytotoxicity is dependant on the DMSO-concentration, the time of exposure and the temperature of the stem cell suspension during the time of exposure to DMSO (9, 10, 11, 12, 13, 14, 15, 16).

Therefore before freezing respectively after thawing the stem cell suspension has to be kept cool at 2°C whilst CryoSure-DEX40 is added to the stem cell suspension, respectively before removal of CryoSure-DEX40 from the stem cell suspension after thawing.

Immediately after addition of DMSO the freezing process has to be started.
Likewise immediately after thawing the wash out process has to be started.

In case of adequate cooling of the stem cell suspension during DMSO-exposure in the unfrozen state, no relevant adverse effects on the cells are observed at end volume concentrations of DMSO between 5 and 10 % (15, 16, 17, 18). Since DMSO is a strong aprotic solvent, special care has to be taken to only use DMSO-compatible materials for withdrawal of the DMSO from the vial and during transition of the DMSO to the target suspension and to minimize the contact time of DMSO with such materials. All processes related to the application and elimination of CryoSure-DEX40 have to be validated by the user.
Addition of CryoSure-DEX40 to the stem cell suspension
Zugabe von CryoSure-DEX40 zur Stammzellsuspension

1. Calculation of the composition of the cryoprotective solution
The amount of CryoSure-DEX40 to be added to the stem cell suspension has to be chosen in a way so that the envisaged endvolume-concentration of DMSO is met. 8 ml of CryoSure-DEX40 contain 4 ml of DMSO (± 4.4 g DMSO). The specific gravity of DMSO is 1.1 g/cm³. In accordance with the protocol of Rubinstein et al., 5 ml of CryoSure-DEX40 are to be added to 20 ml of volume-reduced cord blood. Like this the added 2.5 ml of DMSO result in an endvolume-concentration of DMSO of 10% within the ready-to-freeze suspension (1).

2. Withdrawal of CryoSure-DEX40 from the vial and preparation of the cryoprotective solution
Before addition to the stem cell suspension CryoSure-DEX40 is to be cooled to 2°C. In order to reach the envisaged concentration of CryoSure-DEX40 in the endvolume the necessary amount is to be taken volumetrically from the vial.

3. Addition of the cryoprotective solution to the suspension of hematopoietic stem cells
Before addition of CryoSure-DEX40 the stem cell suspension is placed on an ice bed and cooled to 2°C. Thereafter CryoSure-DEX40, which has also been cooled to 2°C, is added volumetrically at a constant velocity within a period of 15 minutes to the stem cell suspension until the designated end volume is reached. Preferably a calibrated syringe pump is to be used for the addition of the cryoprotective solution (1). The decelerated addition of the DMSO-containing CryoSure-DEX40 provides for the osmotic tolerance of the hyperosmolaric DMSO and the cells in the target suspension. During the addition process the target suspension is continuously and consistently mixed in order to assure a consistent dispersion of the conveyed DMSO within the target suspension (1).

4. Begin of freezing process
Immediately after addition of the complete designated amount of CryoSure-DEX40 to the target suspension the freezing process has to be started. Until the beginning of freezing the temperature of the ready-to-freeze stem cell suspension has to be kept at 2°C. For freezing standard freezing procedures have to be applied as specified in literature. A freezing rate of 1°C/minute until the final freeze-store temperature is reached has been described as an applicable freezing rate for hematopoietic stem cells (16).
Withdrawal of cryoprotectant from the stem cell suspension after thawing

Immediately after the completed thawing process the cryoprotective solution must be washed out of the stem cell suspension. The washing process is executed in several washing steps consisting of centrifugation, withdrawal of liquid supernatant and resuspension of the cells with an appropriate wash solution. During the washing process until the quantitative elimination of the cryoprotectant from the stem cell suspension the suspension has to be kept cool at 2°C. Consequently the wash out process has to be performed by means of a refrigerated centrifuge. The validation of the elimination process is the responsibility of the user. For performing the wash out process within a closed system several methods are available (19-25).
Sources

4) Leibo 1977, Ciba Foundation Symposium No.52, Amsterdam S. 69-96
9) Goris A.: ‘[Test of the toxicity of dimethyl sulfoxide (D.M.S.O.) on carrot tissue cultured in vitro]’, Ann Pharm Fr. 1966 Dec;24(12):781-4
10) Basch, H., and Gadebusch, H.H. ‘Hematopoietic progenitor cells are resistant to dimethyl sulfoxide toxicity’ , Transfusion 34, Nr.10 , 887-890 (1994)
11) Calmels B, Houzé P, Hengesse JC, Ducrot T, Malenfant C, Chabannon C., ‘Clinical evaluation of an automated closed fluid management device: Cytomate, for washing out DMSO from hematopoietic stem cell grafts after thawing’, Bone Marrow Transplant. 2003 May;31(9):823-8