Li StarFish S.r.l.
Via Cavour, 35 - 20063 Cernusco S/N (MI), Italy
Tel. +39-02-92150794 - Fax. +39-02-92157285
info@listarfish.it -www.listarfish.it

CryoSure-DEX40

Cryoprotectant for the cryopreservation of hematopoletic 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

CE 0482

WARNING: Do not autoclave. **Do not use unless solution is clear.** **Not for Injection.**
ACHTUNG: Nicht autoklavieren. **Nur klare Lösung verwenden.** **Nicht injizieren.**

STERILE A — Sterilized by sterile-filtration
Steril durch Anwendung aseptischer Verfahrenstechniken

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

Use until: see product labelling
Verwendbar bis: siehe Produktetikett

Do not re-sterilize.
Nicht erneut sterilisieren.

Protect from strong light.
Vor Sonnenlicht geschützt aufbewahren.

LOT — Lotnumber: see product labelling
Chargennummer: siehe Produktetikett

Do not use if packaging is damaged.
Bei Beschädigung Verpackung nicht verwenden.

Follow instructions for use.
Gebrauchsanweisung beachten.

REF — WAK-DEX40-25 (25 x 8 ml)

Instructions for use CryoSure-DEX40

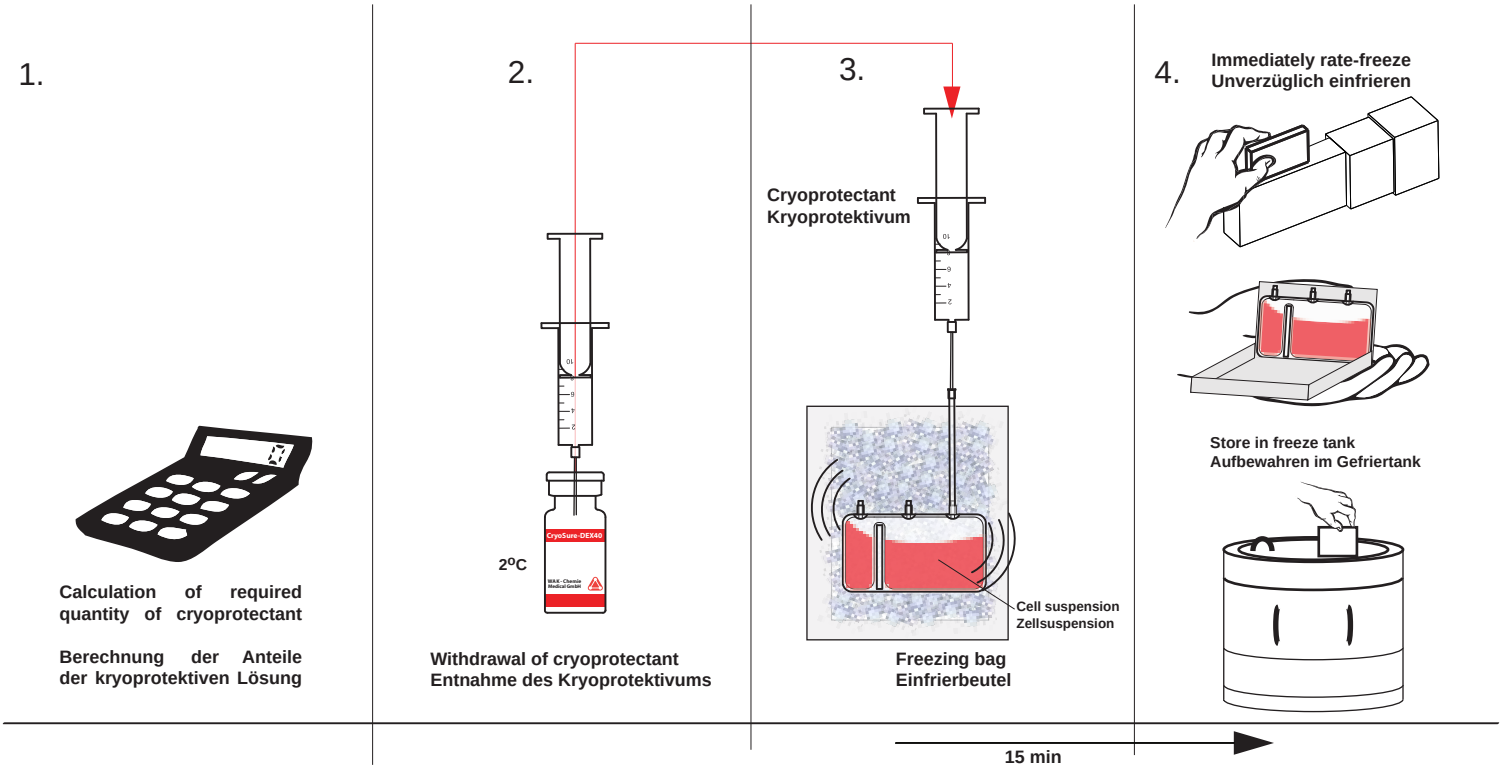
Introduction:
CryoSure-DEX40 is a ready-to-use cryoprotective solution for the addition to a volume reduced buffy coat suspension from cord blood according to the method of Rubinstein et al (1).
CryoSure-DEX40 is a solution consisting of 50 % v/v DMSO and 50 % v/v of a 10% aqueous solution of Dextrane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

I Addition of CryoSure-DEX40 to the stem cell suspension
Zugabe von CryoSure-DEX40 zur Stammzellsuspension



EN

I Addition of CryoSure-DEX40 to the stem cell suspension

CryoSure-DEX40 is added to the volume-reduced cord blood suspension as the last step before initiating the freezing process.

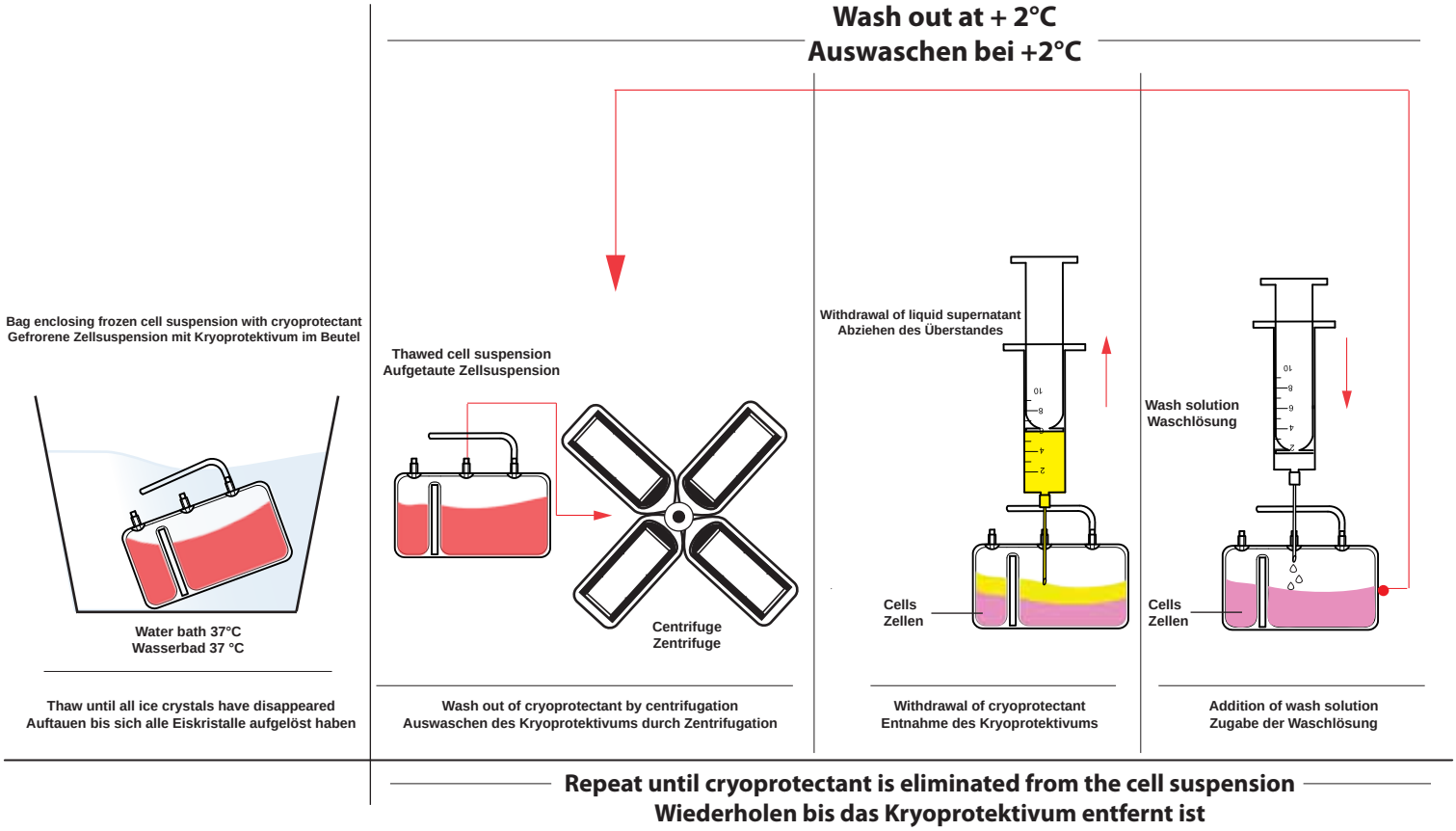
1. Calculation of the composition of the cryoprotective solution
The amount of CryoSure-DEX40 to be added to the stem cell suspension has to be chosen in a way so that the envisaged endvolume-concentration of DMSO is met. 8 ml of CryoSure-DEX40 contain 4 ml of DMSO (≙ 4,4 g DMSO) The specific gravity 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 dispension 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).

II Withdrawal of cryoprotectant from the stem cell suspension after thawing
Entfernung des Kryoprotektivums aus der Stammzellsuspension



EN

II 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 ist the resonsibility of the user. For performing the wash out process within a closed system several methods are available (19-25).

Sources

1) Rubinstein, P.; Dobrila, L.; Rosenfield, R.E.; Adamson, J.W.; Migliaccio, G.; Migliaccio, A.R.; Taylor, P.E.; Stevens, C.E .: "Processing and cryopreservation of placental / umbilical cord blood for unrelated bone marrow reconstitution" . Proc. Natl. Acad. Sci. USA 92 , 10119-10122 (1995)
2) Farrant 1969, Nature, 11755
3) Mazur 1970, Science 168, 939-949
4) Leibo 1977, Ciba Foundation Symposium No.52, Amsterdam S. 69-96
5) Merryman et al 1977 Cryobiology 14, 287-302).
6) Whittingham 1981, Gustav-Fischer Verlag, Stuttgart, S. 21-22
7) Lovelock 1953, Biochim. Biophys. Acta 10, 414-426
8) Merryman 1974, An. Rev. Of Biophys. And Bioengineering, Band 3 Paolo Alto, S.341-363
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. In vitro antimicrobial activity of dimethyl sulfoxide. Appl. Microbiol. 16: 1953-1954 (1968).
11) Chang CY, Simon E: 'The effect of dimethyl sulfoxide (DMSO) on cellular systems.', Proc Soc Exp Biol Med. 1968 May;128(1):60-6
12) Da Violante G, Zerrouk N, Richard I, Provot G, Chaumeil JC, Arnaud P: ,Evaluation of the cytotoxicity effect of dimethyl sulfoxide (DMSO) on Caco2/TC7 colon tumor cell cultures', Bilo. Pharm. Bull. 25(12) 1600-1603 (2002)
13) Fahy GM: 'The relevance of cryoprotectant ''toxicity'' to cryobiology.', Cryobiology 1986 Feb;23(1):1-13
14) Gurtuvenko AA, Anwar J: 'Modulating the structure and properties of cell membranes: the molecular mechanism of action of dimethyl sulfoxide.', J Phys Chem B. 2007 Sep 6;111(35):10453-60
15) Yang H, Zhao H, Acker JP, Liu JZ, Akabutu J, McGann LE: 'Effect of dimethyl sulfoxide on post-thaw viability assessment of CD45+ and CD34+ cells of umbilical cord blood and mobilized peripheral blood.', Cryobiology 2005 Oct;51(2):165-75
16) Hunt CJ, Armitage SE, Pegg DE: 'Cryopreservation of umbilical cord blood: 2. Tolerance of CD34(+) cells to multimolar dimethyl sulphoxide and the effect of cooling rate on recovery after freezing and thawing.', Cryobiology. 2003 Feb;46(1):76-87
17) Rowley S.D., Anderson G.L.: 'Effect of DMSO exposure without cryopreservation on hematopoietic progenitor cells'. Bone Marrow Transplant.11, 389-393 (1993)
18) Branch, D.R.; Calderwood, S.; Cecutti, M.A.; Herst, R.; Solh, H .: "Hematopoietic progenitor cells are resistant to dimethyl sulfoxide toxicity" . Transfusion 34, Nr.10 , 887-890 (1994)
19) Calmels B, Houzé P, Hengesse JC, Ducrot T, Malenfant C, Chabannon C.: , Preclinical 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.
20) Rodríguez L, Velasco B, García J, Martín-Henao GA.: 'Evaluation of an automated cell processing device to reduce the dimethyl sulfoxide from hematopoietic grafts after thawing.', Transfusion. 2005 Aug;45(8):1391-7.
21) Foïs E, Desmartin M, Benhamida S, Xavier F, Vanneaux V, Rea D, Fermand JP, Arnulf B, Mounier N, Ertault M, Lotz JP, Galicier L, Raffoux E, Benbunan M, Marolleau JP, Larghero J.: 'Recovery, viability and clinical toxicity of thawed and washed haematopoietic progenitor cells: analysis of 952 autologous peripheral blood stem cell transplantations.', Bone Marrow Transplant. 2007 Nov;40(9):831-5. Epub 2007 Aug 27.
22) Laroche V, McKenna DH, Moroff G, Schierman T, Kadidlo D, McCullough J.: , Cell loss and recovery in umbilical cord blood processing: a comparison of postthaw and postwash samples.', Transfusion. 2005 Dec;45(12):1909-16.
23) Lemarie C, Calmels B, Malenfant C, Arneodo V, Blaise D, Viret F, Bouabdallah R, Ladaique P, Viens P, Chabannon C.: 'Clinical experience with the delivery of thawed and washed autologous blood cells, with an automated closed fluid management device: CytoMate.', Transfusion. 2005 May;45(5):737-42.
24) Nagamura-Inoue T, Shioya M, Sugo M, Cui Y, Takahashi A, Tomita S, Zheng Y, Takada K, Kodo H, Asano S, Takahashi TA.: 'Wash-out of DMSO does not improve the speed of engraftment of cord blood transplantation: follow-up of 46 adult patients with units shipped from a single cord blood bank.', Transfusion. 2003 Sep;43(9):1285-95.
25) Perotti CG, Del Fante C, Viarengo G, Papa P, Rocchi L, Bergamaschi P, Bellotti L, Marchesi A, Salvaneschi L.: 'A new automated cell washer device for thawed cord blood units.', Transfusion. 2004 Jun;44(6):900-6.