

VitriStore Freeze Kit

Material included in the package:

- Product code: 4 VF -KIT1
- 1 x 5 ml VitriStore Pre-vitrification Medium (4 VFPI-005)
- 1 x 1 ml VitriStore Thaw Medium 1 (4 VF1001)
- 1 x 1 ml VitriStore Thaw Medium 2 (4 VF2001)

VitriStore Thaw Kit

Material included in the package:

- Product code: 4 VT -KIT1
- 1 x 5 ml VitriStore Thaw Medium 1 (4 VT1001)
- 1 x 1 ml VitriStore Thaw Medium 2 (4 VT2001)
- 1 x 1 ml VitriStore Thaw Medium 3 (4 VT300)
- 1 x 1 ml VitriStore Thaw Medium 4 (4 VT400)

The media should be used in the order displayed above.

Material not included in the package:

- 4-well dishes
- Freezer tank with liquid nitrogen
- Water bath (able to hold 37°C)
- Pipettes
- Forceps
- Vitrification device
- LAF bench (ISO 5 environment)
- Microscope
- Lab timer

Product specifications and quality control:

- All raw materials are of highest available purity (European Pharmacopoeia and/or USP standard) if applicable.
- A certificate of analysis is provided for each batch upon request from our laboratories with lot number.
- The MSDS for the media are available upon request and can also be downloaded from our website.
- GM501 VitriStore Freeze/VitriStore Thaw are manufactured and tested according to the following specifications:

pH 7.0-7.40

Osmolality (mOsm/kg)
270-290 (VitriStore Freeze 1)
805-850 (VitriStore Thaw 1)
535-565 (VitriStore Thaw 2)
405-435 (VitriStore Thaw 3)
270-290 (VitriStore Thaw 4)

Sterility sterile - SAL10⁻³ (Sterility Assurance Level)

Endotoxins (EU/ml) < 0.25

Mouse Embryo Assay (MEA) ≥80
(1-cell assay, blastocysts after 96 h in %)

EN: Intended use/intended users:

- GM501 VitriStore Freeze/VitriStore Thaw are a set of ready-to-use media for vitrification and thawing of human embryos.
- The intended users are IVF professionals (lab technicians, embryologists or medical doctors).

Composition:

- GM501 VitriStore Freeze/VitriStore Thaw are DMSO/ethylene glycol based vitrification media also contain PBS, sucrose, Ficoll and human serum albumin (10-20 g/liter).
- GM501 VitriStore Freeze/VitriStore Thaw do not contain antibiotic.

Instructions for use:

- Ensure all media are well mixed before use. We strongly advise to read through all the steps of the vitrification/warming procedure before starting the procedure.

Preliminary steps

- Fill the 4-well dish with the first well with 200 µl of VitriStore Pre-vitrification Medium, the second with 250 µl VitriStore Freeze Medium 1 and the third with 250 µl VitriStore Freeze Medium 2. Next open as many packs of vitrification devices as will be required for the vitrification step. Conveniently place the separate parts of the vitrification device on the workbench for easy access later in the procedure.

Up to 5 vitrification cycles (of same patient) can be performed with one media set-up. Do not use the same media for different patients!

Freezing preparation

Transfer the embryos from the blastocyst cell culture medium into each of the VitriStore Freeze solutions using the following scheme:

Stage	Vitri Store Pre-vitrification	Vitri Store Freeze 1	Vitri Store Freeze 2	Temperature
Early Blastocyst, Morulae	2 min.	2 min.	30 sec.	Room temperature
Blastocyst-expanded Bl.	2 min.	3 min.	30 sec.	37°C
Blastocyst-expanded Bl + artificial shrinkage*	2 min.	2 min.	30 sec.	Room temperature

*Before starting the vitrification procedure, in order to reduce the negative effect of the blastocell, expanded blastocysts should be collapsed by reducing with a glass pipette the volume of the blastocyst. (Vanderzwalmen et al., 2002; Son et al., 2003; Hiraoka, 2004)

Vitrification:

1. Using an attorneyed pipette or an equally suitable device, deposit maximum 2 blastocysts in a volume of approximately 0.3 µl of VitriStore Freeze 2 in the gutter of the vitrification straw.
2. Place the vitrification straw in the outer sheath and seal it as indicated in the instructions for use of the vitrification device.
3. Plunge the sealed device into the liquid nitrogen.

Thawing:

1. In a 4-well dish, fill the first well with 1 ml VitriStore Thaw Medium 1, the second with VitriStore Thaw Medium 2 (250-300 µl). Preheat the media to 37°C for 10 minutes.
2. Remove the vitrification straw from the outer sheath and seal it as indicated in the instructions for use of the vitrification device.
3. Plunge the sealed device into the liquid nitrogen.

Re-thawing:

1. Transfer into VitriStore Thaw Medium 2 (37°C) and leave in this medium for 2 minutes.
2. Transfer into VitriStore Thaw Medium 3 (37°C) and leave in this medium for 2 minutes.
3. Finally transfer into VitriStore Thaw Medium 4 (37°C) and wash for at least 1 minute.

7. Transfer into blastocyst culture medium for continued cell culture.

Re-thawing:

For re-thawing, use the same scheme as for the initial freezing.

Instructions for use:

- All media are well mixed before use. We strongly advise to read through all the steps of the vitrification/warming procedure before starting the procedure.

Preliminary steps

- Fill the 4-well dish with the first well with 200 µl of VitriStore Pre-vitrification Medium, the second with 250 µl VitriStore Freeze Medium 1 and the third with 250 µl VitriStore Freeze Medium 2. Next open as many packs of vitrification devices as will be required for the vitrification step. Conveniently place the separate parts of the vitrification device on the workbench for easy access later in the procedure.

Up to 5 vitrification cycles (of same patient) can be performed with one media set-up. Do not use the same media for different patients!

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Re-thawing:

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Frühe Blastozyste, Morulae	2 Min.	2 Min.	30 sec.	Raumtemperatur
Blastozyste -exp. Bl.	2 Min.	3 Min.	30 sec.	37°C
Blastozyste -exp. Bl + artifizielle Schrumpfung*	2 Min.	2 Min.	30 sec.	Raumtemperatur

*Um negative Auswirkungen zu reduzieren, sollte bei expandierten Blastozysten das Blaszkotest mit Hilfe eines Glasstempel körnig kolabiert werden. (Vanderzwalmen et al., 2002; Son et al., 2003; Hiraoka, 2004)

Einheiten:

- 1. Mit einer alternierenden Pipette oder einer ebenso geeigneten Vorrichtung bringen 2 Blastozysten in einem Volumen von ca. 0,3 ml VitriStore Freeze media in die Rinne der Spritze des Vitrifizierungshahmen geben.

Die Emulsion aus dem Kultivierungsmedium in folgender Reihenfolge und Dauer in jeder der VitriStore Freeze-Lösungen überführen:

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