Suppl. Fig. 1 | Vitrification Kit and Warming and Recovery Kit Instructions

the Oncofertility® Consortium

Human Ovarian Tissue Vitrification Kit

These products are for research use only.

INTENDED USE

These products are intended for the ultra-rapid vitrification of human ovarian tissue. All media preparations and procedures must be carried out in a biosafety cabinet.

MATERIALS PROVIDED IN THE VITRIFICATION KIT

1 x 11 ml bottle of Equilibrium Solution 1 (ES1)
1 x 16 ml bottle of CryoMedium (CM)
1 x 19 ml bottle of Vitrification Solution (VS)
1 x 17 ml bottle of Vitrification Solution with Polymers (VS+PXZ)
1 x 2 ml vial containing 29 mg lyophilized ascorbic acid
2 x 30 ml empty sterile bottles labeled ES2 and ES3
3 x 2 ml high security tissue straws
Sterile absorption spears

PRECAUTIONS AND WARNINGS

Caution: The user should read and understand all directions for use, precautions and warnings before using Vitrification Kit.

Caution: Handle all human source material using universal precautions. Wear safety goggles and face mask.

Single Use: Kit should be used for one patient. Discard any excess product that remains after procedure is complete.

QUALITY ASSURANCE

The reagents in this kit are membrane filtered and aseptically processed according to GMP procedures, which have been validated to meet a sterility assurance level (SAL) of 10^{-3}. A certificate of analysis is included.

MATERIALS REQUIRED BUT NOT INCLUDED

20-27 gauge needle and syringe
P1000 pipette with sterile tips
P20 pipette with sterile tips
Forceps
60 mm petri dishes
Liquid nitrogen and dewar flask
Slide warmer
Tube rack
Heat sealer
Goblets for straw storage
Stackable canisters for liquid nitrogen storage

DIRECTIONS FOR USE

Note: Make all media fresh. Once ascorbic acid is added to solutions, solutions must be used within 24 hours. Label with date and time of preparation.

Media Preparation:

1. Prepare 2 ml of 100 μM ascorbic acid by using a 20-27 gauge needle and syringe to pierce ascorbic acid vial lid and add 2 ml of CM. Vortex to dissolve.
2. Using a P20 pipette with sterile tips, add the following volumes of 100 μM ascorbic acid to each of the provided media for a final concentration of 100 nM.

Media	Volume of ascorbic acid
ES1	11 μl
CM	14 μl
VS	19 μl
VS+PXZ	17 μl

3. Make 10 ml of Equilibrium Solutions 2 and 3 (ES2, ES3) in the provided sterile bottles by combining VS and CM according to the chart below.

Media	VS	CM	Total
ES2	2.5 ml	7.5 ml	10 ml
ES3	5 ml	5 ml	10 ml

4. Make a liquid nitrogen vapor environment using liquid nitrogen, a dewar flask, or suitable liquid nitrogen container, and tube rack. The tube rack should be high enough that tissue straws will not be submerged, and only exposed to vapor.
5. Label 5 - 60 mm petri dishes: ES1, ES2, ES3, VS, VS+PXZ. Arrange dishes on a slide warmer in a biosafety cabinet.
6. Transfer 10 ml of ES1, ES2, ES3, VS, and VS+PXZ to their respective plates, allow media to warm for 15 minutes, and then proceed to media validation test.

Media Validation Test:

1. Heat seal one end of a 2 ml high security tissue straw by putting the straw between seal bars and pressing down the heat sealer for ~3 seconds. Repeat 3 times.

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2. Pipet up and down to mix thoroughly, and then pipet 2 ml VS+PXZ into the straw.
3. Use a sterile absorption spear to dry the inside of the straw near the open end to ensure efficiency of the heat seal.
4. Heat seal straw at the open end.
5. Place the straw in the liquid nitrogen vapor in closed dewar flask for at least 10 minutes. Do not submerge in liquid nitrogen at this time.
6. Open lid and confirm that the solution vitrified. Color should be clear/transparent, not white, and there should be no ice crystals. If solution appears cloudy, stir VS+PXZ thoroughly and repeat media validation test.

Vitrification Procedure:

1. Before starting vitrification procedure, heat seal one end of high security 2 ml tissue straw. Pipet 2 ml of VS+PXZ into each straw. Each straw will hold up to 10 pieces of tissue (5 x 5 x 0.5mm).
2. Using forceps, transfer tissue pieces to ES1 and incubate at 37°C on slide warmer, swirling plate 2 times every 30 seconds for 7 minutes.
3. Transfer tissue pieces to ES2 and incubate at 37°C on slide warmer, swirling plate 2 times every 30 seconds for 7 minutes.
4. Transfer to ES3 and incubate at 37°C on slide warmer, swirling plate 2 times every 30 seconds for 7 minutes.
5. Transfer to VS and incubate at 37°C on slide warmer, swirling plate 2 times every 30 seconds for 7 minutes.
6. Transfer to VS+PXZ and incubate at 37°C on slide warmer for 30 seconds, swirling twice, then immediately proceed to tissue loading.
7. Using forceps, transfer tissues into preloaded straws, up to 10 pieces in each straw. Wait for tissue to sink into media before loading the next piece, so that there is a little space between each tissue piece. Use a sterile stick, cryoloop, or pipet tip to gently push down tissue pieces if they get stuck on the walls of the straw.
8. After loading all tissue pieces, use a sterile absorption spear to dry the inside of the straw near the open end to ensure efficiency of heat seal.
9. Heat seal open end of the straw.
10. Place sealed straw into liquid nitrogen vapor (on top of a tube rack), being careful not to submerge.
11. Cool for 10 minutes in the liquid nitrogen vapor, and then immediately plunge in liquid nitrogen.
12. Place sealed straws inside goblets, and goblets inside stackable canisters. Store in liquid nitrogen (liquid phase).

STORAGE INSTRUCTIONS AND STABILITY

Store Human Ovarian Tissue Vitrification Kit at 4°C for up to one year prior to use. Do not add ascorbic acid to the kit components until day of vitrification. Once ascorbic acid has been added to solutions, use within 24 hours.

REFERENCES

1. Ting AY, Yeoman RR, Campos JR, Lawson MS, Mullen SF, Fahy GM, Zelinski MB. Morphological and functional preservation of preantral follicles after vitrification or macaque ovarian tissue in a closed system. Hum Reprod, 28:1267-79, 2013 PMID: 23427232 PMCID: PMC3627338.
2. The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril. 99:37-43, 2013.

EXPLANATION OF SYMBOLS

	Catalog Number
	Batch Number
	Use By (year, month, day)
	Do not reuse
	Temperature Limitation
	Aseptic Technique Sterilization Membrane Filtered (SAL 10^{-3})
	Attention: See instructions for use
	Manufacturer