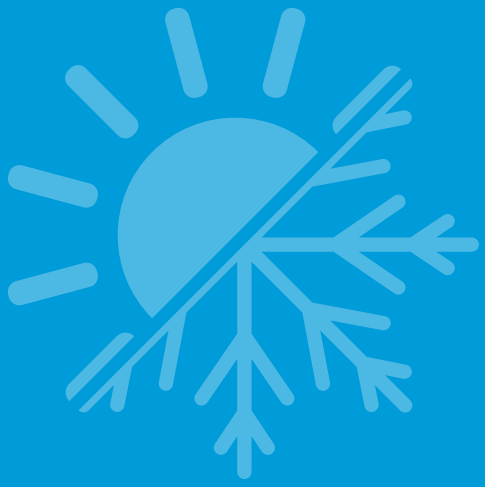
VITROMED
Germany

2025



V-VIT WARM/FREEZE Application Handbook

V-VIT FREEZE V-VIT WARM

This is an additional protocol sheet. Please refer to the Instructions for Use supplied with the product or available online for any information/warning/explanation.

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PRODUCT DESCRIPTION

V-VIT FREEZE is a ready-to-use kit to cryopreserve human oocytes and embryos (up to blastocyst stage) in Assisted Reproduction Technology (ART). There are in total 3 vials of pink solutions in the package:

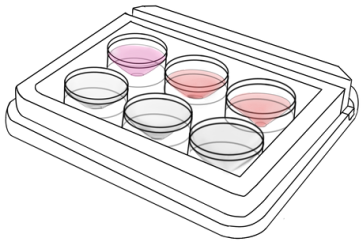
- 1 vial of Equilibration Solution (ES), with a blue cap and label.
- 2 vials of Vitrification Solution (VS), with green caps and labels.

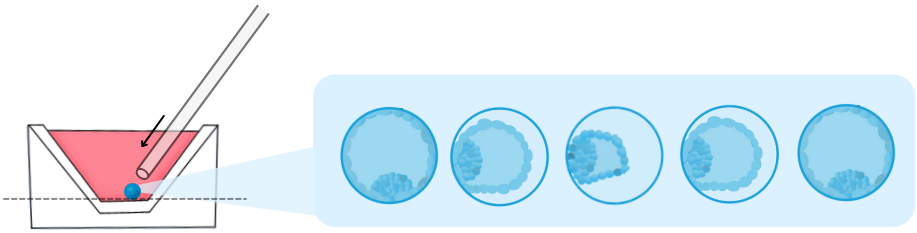
PREPARATION BEFORE USE

- The whole process should be performed at room temperature.
- ES and VS should be at room temperature when vitrification starts.
- Liquid nitrogen is needed for vitrification.
- Compare the thickness of the perivitelline space with the thickness of the zona pellucida, and record the comparison.
- Use a sterilized pipette with appropriate diameter to handle oocytes (recommended 120 – 150 μm) and blastocysts (recommended 180 – 300 μm).
- Maximum 3-4 oocytes (MII) or pronuclear (PN) zygotes can be processed using these procedures before needing to replace medium.
- Maximum 1-2 embryos can be processed using these procedures before needing to replace medium.

INSTRUCTIONS FOR USE

1. Add 200 μl ES to the first well of a vitrification plate, and add 200 μl VS to the second and third well.
2. Place an oocyte(s)/embryo(s) at the centre of the surface of the ES liquid in the first well.
3. The oocyte/embryo will slowly sink to the bottom of the well. Leave the oocyte and the embryo at the blastocyst stage in the ES liquid for 15 min, while leave the embryo at other stages in the liquid for 12 min. During this process, the oocyte/embryo will start to shrink, and then return to its original size.

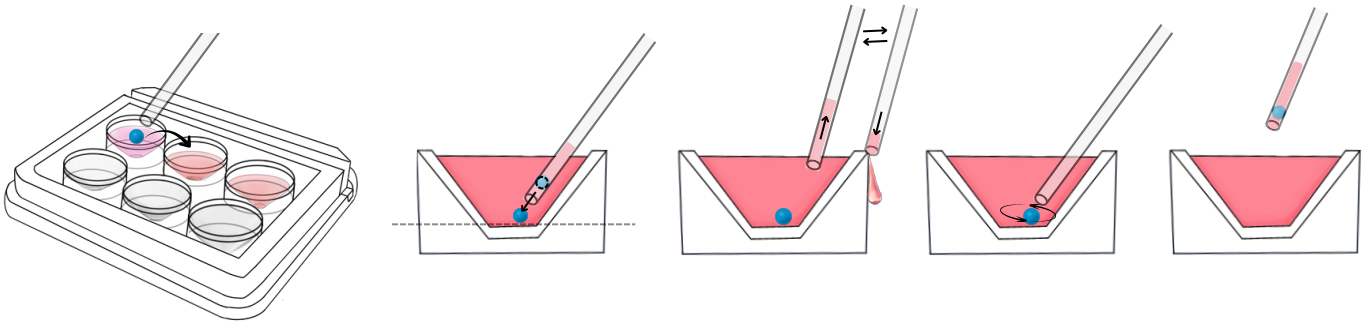




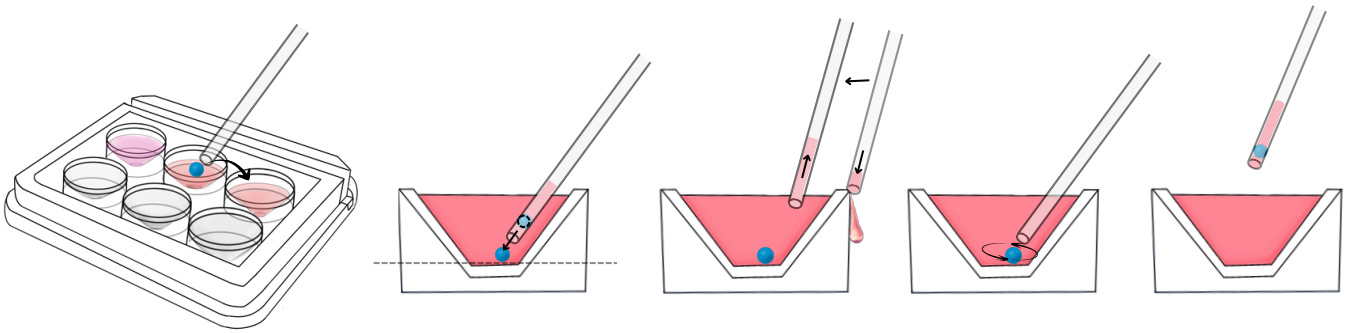
Shrinkage and re-expansion of embryo

Caution: Step 4 to Step 6 should be completed within 30 to 40 sec.

4. Transfer the oocyte/embryo from the first well to the second well using a pipette. Discard the remaining ES in the pipette tip. Rinse the pipette tip by aspirating in V-VS from the second well and discarding the taken-in V-VS. Gently move the pipette around the oocyte/embryo.
5. Change the position of the oocyte/embryo in the same well. Gently move the pipette around the oocyte/embryo.
6. Repeat step 5 again.



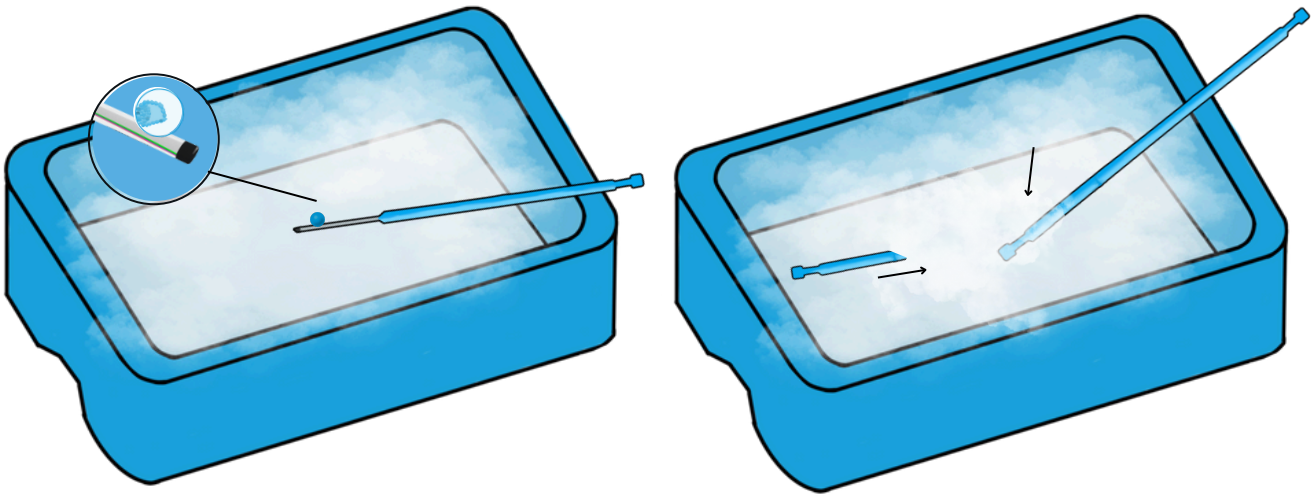
7. Rinse the pipette tip by intaking VS from the third well and discarding the VS in the pipette.
8. Transfer the oocyte/embryo from the second well to the third well. Take as little as possible VS from the second well during the transfer. Gently move the pipette around the oocyte/embryo after transfer.
9. Change the position of the oocyte/embryo in the same well. Gently move the pipette around the oocyte/embryo.



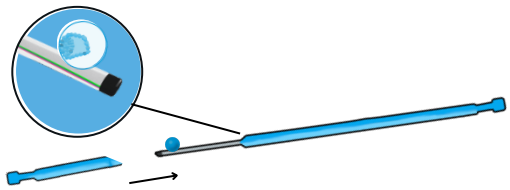
Caution: Step 7 to Step 9 should be completed within 20 to 30 sec.

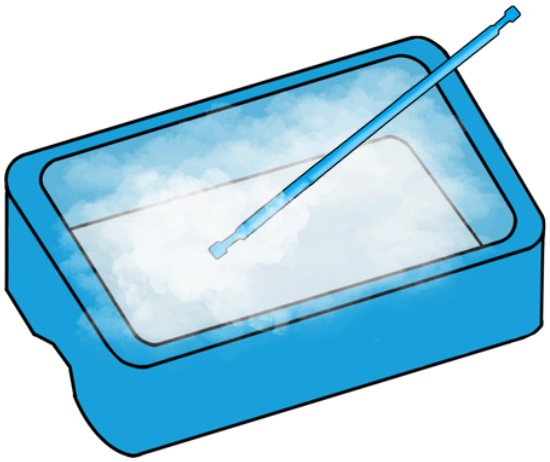
10. Place the oocyte/embryo on the suitable labelled carrier device (Note: Users in USA should use a closed cryopreservation device that is cleared in the US). Minimize the volume of the VS around the oocyte/embryo on the device (< 0.1 µl).

Open Device:
11. Immerse the non-capped device in liquid nitrogen, pre-equilibrate the cap also in liquid nitrogen and close the device with the cap under liquid nitrogen



Closed device usage:
11. Cover the device with a cap, and then immerse the capped device in liquid nitrogen.





12. Store the carrier device in a storage tank.

V-VIT WARM

PRODUCT DESCRIPTION

V-VIT WARM is a ready-to-use kit to thaw human oocytes and embryos (up to blastocyst stage) which are cryopreserved. Subsequently, the thawed oocytes and embryos can be used in Assisted Reproduction Technology (ART) processes. There are in total 4 vials of pink solutions in the package:

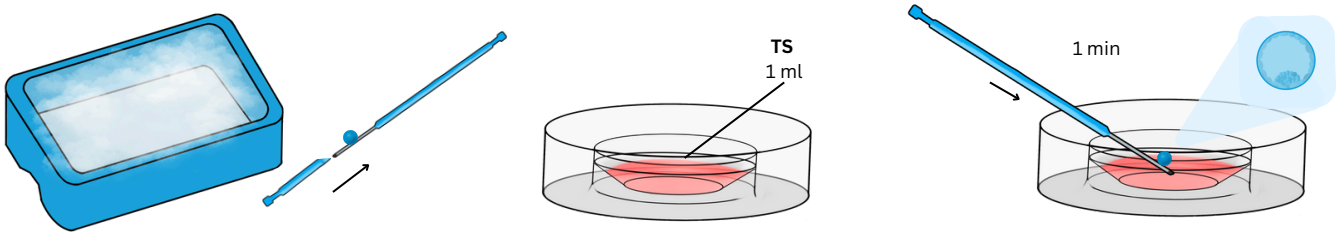
- 2 vials of Thawing Solution (V-TS), with red caps and labels.
- 1 vial of Diluent Solution (V-DS), with a yellow cap and label.
- 1 vial of Washing Solution (V-WS), with a white cap and label.

PREPARATION BEFORE USE

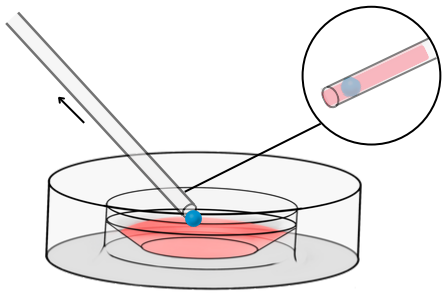
- The whole process should be performed at room temperature unless otherwise indicated.
- Reverse the vials to mix the solutions before use. Shake the vials down using quick, sharp, downward wrist motions after reversing the vials to avoid liquid sticking in the inner side of the caps.
- Incubate the TS vial (closed) and a petri dish in an incubator at 37 °C for at least 1.5 h.
- DS and WS should be at room temperature when thawing procedure starts.
- Use a sterilized pipette with appropriate diameter to handle oocytes (recommended 120 – 150 µm) and blastocysts (recommended 180 – 300 µm).
- Keep the cryopreserved oocytes or embryos frozen in liquid nitrogen, before the thawing procedure starts.
- Maximum 3-4 oocytes (MII) or pronuclear (PN) zygotes can be processed using these procedures before needing to replace medium.
- Maximum 1-2 embryos can be processed using these proce dures before needing to replace medium.

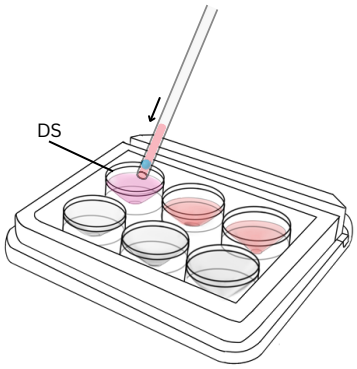
INSTRUCTIONS FOR USE

1. Take a sterile multi-well plate, and add 200 µl DS to the 1st well.
2. Take the Petri dish and TS vial out of the incubator, and add 1 ml to the Petri dish.
3. Take out one carrier device with a frozen oocyte/embryo from the cooling device (e.g., liquid nitrogen tank). (Note: Users in USA should use a closed cryopreservation device that is cleared in the US.) Within 1 sec, put the terminal of the device with the oocyte/embryo into the Petri dish with TS and incubate in the TS for 1 min. Make sure that the covering cap of the carrier device has been removed before placing the device into the TS. Make sure that the oocyte/embryo is immersed in the TS.

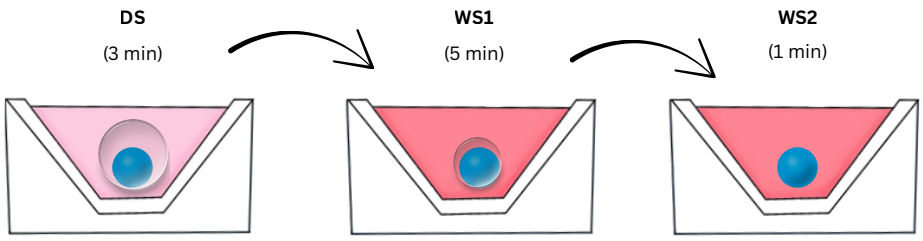


4. When in the TS, the oocyte/embryo naturally separates from the carrier device and start to float.
5. Aspirate the oocyte/embryo into a sterile pipette. After the oocyte/embryo is in the pipette, in addition, aspirate enough extra TS into the pipette (about 3 mm in length be low the oocyte/embryo)
6. Insert the tip of the pipette into the centre bottom of the 1st well of the multi-well plate the with DS (prepared in step 1). Expel TS slowly to make a TS layer at the bottom of the well. Carefully place the oocyte/embryo into the bottom of the TS layer. Wait for 3 min.

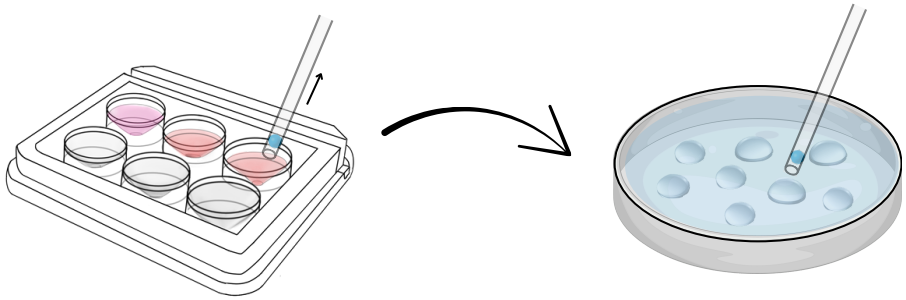


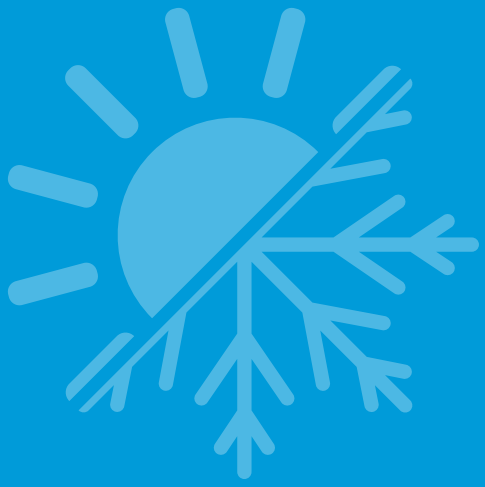


7. During the waiting period, fill the 2nd and the 3rd wells with 200 µl WS.
8. Aspirate the oocyte/embryo in a sterile pipette, and then slowly aspirate extra DS in the pipette (about 3 mm in length below the oocyte/embryo).
9. Insert the tip of the pipette into the centre bottom of the 2nd well of the multi-well plate the with WS (prepared in step 7). Expel DS slowly to make a DS layer at the bottom of the well. Carefully place the oocyte/embryo into the bottom of the DS layer. Wait for 5 min.
10. After 5 min, observe the shape of the oocyte/embryo, if the shrunken oocyte/embryo returns to the original volume, it can be proceeded further.
11. Aspirate the oocyte/embryo in the pipette and transfer it to the 3rd well with WS. Place the oocyte/embryo at the surface of the WS and let the oocyte/embryo naturally sink to the bottom.
12. Take the oocyte/embryo again to the pipette, and place the oocyte/embryo at a different location of the surface of the WS in the same well. Let the oocyte/embryo naturally sink to the bottom.



13. Transfer the oocyte/embryo to a culture dish with appropriate culture medium. Assess the survival and proceed with further steps.





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