Nexpring Health

NEW Blastocyst Fast

Vitrification Protocol with Vit Kit - Freeze NX

The following protocol is for use of Vit Kit - Freeze NX (PN 90188) with blastocyst stage embryos and your vitrification/cryostorage device of choice. Please familiarize yourself with proper handling of the device. Vit Kit - Freeze NX contains Equilibration NX - ES (ES), Vitrification NX - VS (VS), and Washing NX - WS (WS). Procedures must be performed at room temperature (20–27ºC). It is advised to minimize exposure of specimen to light during equilibration in ES and VS solutions. Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure.

INITIAL PREPARATION

01 Bring the quantity to be used of ES and VS to room temperature (20–27°C).
Avoid bringing the entire vials of ES and VS to room temperature repeatedly when a partial of the solution is needed each time. It is better to aliquot the quantity to be used and return the vials to 2–8°C right after aliquoting.

02 Fill a liquid nitrogen reservoir with liquid nitrogen (LN2) – sufficient to achieve a depth of 4 inches or to completely submerge cryotube on cane – and place close to microscope. Attach a cryotube or goblet (uncapped) to the bottom clamp of a cryocane and submerge in the liquid nitrogen in preparation for storage of the vitrified specimens.

03 Determine the number of specimens to be vitrified.

04 Label each sterile Petri dish (bottom) and vitrification device with necessary information.

05 Carefully examine the vitrification device before starting procedure.

06 Gently invert each vial of ES and VS to mix contents before use.

BLASTOCYST FAST VITRIFICATION PROTOCOL

07 Aseptically dispense one 50 µL drop of ES on an open dish or inverted lid of Petri dish **(Figure 1)**.

08 Remove the culture dish with embryo(s) from the incubator and check the quality of the specimen(s) under microscope. Where possible, select only the best quality embryo(s) for vitrification.

09 Carefully transfer the specimen (up to two at one time) with a minimal volume of medium from the culture dish to the drop of ES and start the timer.

10 Blastocysts should equilibrate in the ES drop slowly by free-fall for 1 -10 minutes.

Minimize the exposure of specimen(s) to light during equilibration in ES and VS drops.

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11 During this equilibration time in ES, aseptically dispense one 50 µL drop of VS solution as shown in **Figure 1** and prepare vitrification device of choice for loading.

12 Rinse and fill the transfer pipette tip with VS immediately before equilibration in ES is complete, and draw up the specimen(s) with minimal volume of ES into the pipette tip and transfer into the drop of VS for a minimum of 30 seconds. Unload blastocysts to the bottom of VS. While unloading, blastocysts will float to the top of VS. To ensure complete rinse with VS, gently move the blastocysts back to the bottom center of VS by pipetting.

During this process, blastocysts will be dehydrated and float back again.

13 Load and seal the vitrification device as directed by manufacturer.

14 Place the vitrified specimen(s) on the vitrification device of choice into the submerged LN2 filled cryotube or goblet (on the cryocane) **(Figure 2)**. Cap the cryotube or goblet, or attach upside down with another uncapped cryotube in order to secure the vitrified device in liquid nitrogen.

15 Move the LN2 reservoir close to the LN2 cryotank and transfer the cryocane with contents to the cryotank for long-term storage.

Figure 1:

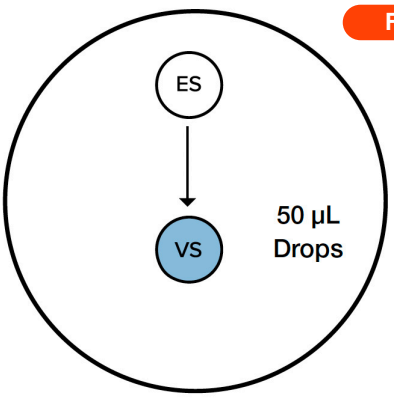
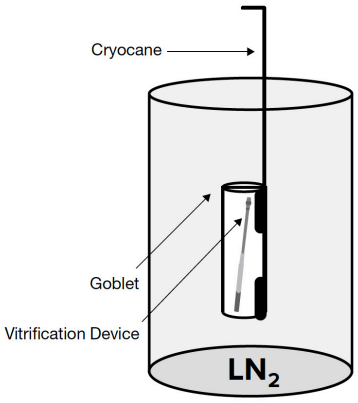


Figure 2:



For additional details on the use of these products, each laboratory should consult its own laboratory procedures and protocols, which have been specifically developed and optimized for your individual medical program.

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TIPS for Blastocyst Vitrification

01 While waiting for exposure time in ES and VS, cover the dish and move away from the light source.

02 Transfer specimens between drops using a minimal volume of medium.

03 After 10 minutes in ES, if the blastocyst has not re-expanded at least 80%, consider moving forward with vitrification process and note the lack of re-expansion.

04 Consider dispensing the VS drop later, such as towards the end of ES exposure, to minimize any risk of evaporation of VS.

05 Consider moving the blastocyst around in VS until it is no longer floating. This is an indication the embryo is fully equilibrated and there is no carryover of ES in the specimen. This process might look like:

- Release blastocyst at the 12 o'clock position into the VS drop and start the 30 second timer. Embryo will float to the top of VS.
- After 10 seconds, gently move the blastocyst to the 6 o'clock position in the VS drop until the end of the 30 second countdown.

06 The timing for exposure to VS is CRITICAL.

- Maintain microscopic visualization of specimen(s) by adjusting focus as needed, during rapid exposure to VS (specimens will float in the drop).
- Keep transfer pipette tip close to drop for quick manipulations.
- Load, seal and plunge the vitrification device within **80 seconds, not to exceed 110 seconds** after initial exposure to VS.

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