



Simplified Oocyte Vitrification Protocol

For MII Oocytes - Gradual Exposure to ES

ALL PROCEDURES MUST BE PERFORMED AT ROOM TEMPERATURE (22-27°C)

As referenced in clinical literature, we recommend oocyte vitrification to be performed within 2 hours from time of oocyte retrieval.

As an added precaution during the preparation procedure, carefully examine each CryoTip outside of the package. Prior to use, all CryoTips should be examined under a suitable magnification (40x power) for possible damage (such as tip breakages or cracks) that may have occurred during transport.

Have all necessary materials, tools and equipment ready and easily accessible before starting procedure.

- 1. Aseptically dispense:
 - one (1) 20 µL drop of H
 - three (3) 20 µL drops of ES (ES1, ES2 and ES3)
 - ES1 and ES2 should be in close proximity to H (but not touching).
- Place MII oocyte(s) (2 maximum), into H and expose undisturbed for 1 minute.
- Merge ES1 with H. Allow spontaneous mixing for 2 minutes.
 Use tip of transfer pipette to move ES1 towards H until drops merge.
- 4. Then merge ES2 with H+ES1. Allow spontaneous mixing for 2 minutes
- Transfer oocyte(s) from merged drop to ES3 and expose undisturbed for 6–10 minutes.

- 6. During the 6–10 minute exposure, aseptically dispense one (1) 50 µL drop of VS.
 - ♥ Visually observe re-expansion (equilibration) of oocyte(s) to at least 80% of original volume.
- 7. Transfer oocyte(s) from ES3 to VS for 30 seconds before loading.
- Gently but thoroughly pipette oocyte(s) once within VS drop to ensure complete rinse in VS.
 - □ To minimize floating, after 10 seconds pipette the specimen(s) to the bottom of the VS drop.
- 9. Load, seal and plunge CryoTip within 80 seconds, not to exceed 110 seconds after initial exposure to VS.
- Refer to CryoTip Loading Protocol, IS P/N 10068ART diagram and product insert for detailed loading instructions.
 - See reverse side for tips.

ES1 2 min ES2	
2 min 1 min ES3 6-10 min	20 µL Drops
30 seconds (pipette once to rinse) Load, seal and plunge the within 110 sec of exposure	50 μL Drop CryoTip e to VS

KEY	
Н	HEPES buffered medium with protein (eg. mHTF- HEPES with 20% SSS or equivalent)
ES	Equilibration Solution
VS	Vitrification Solution
←→	Merge drops
→	Transfer specimen to next drop

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Tips

 All procedures are to be done at ROOM TEMPERATURE (22–27°C).

Do not use heated stage.

- Have all necessary material, tools and equipment ready and easily accessible before starting procedure.
- CryoTips should be pre-labeled with patient information, and assembled with connector and syringe or pipette (for loading), prior to starting procedure. To protect the finely pulled tip from damage, keep it covered with metal cover sleeve until ready to load specimen(s).
- Where possible, select only the best quality MII oocytes for vitrification.
- The recommended CryoTip capacity is a MAXIMUM of 2 specimens. Process only as many specimen(s) as will be loaded per CryoTip at one time.

- Minimize exposure of specimens to light during equilibration in ES and VS solutions.
- Transfer specimens between drops using a minimal volume of medium.
- 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 CryoTip within 80 seconds, not to exceed 110 seconds after initial exposure to VS.

1. Song WY, Sun YP, Jin HX, et al. Chinese Journal of Obstetrics and Gynecology 2010; 45(8):578-82.

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