

Simplified Embryo Vitrification Protocol




2PN to Blastocyst

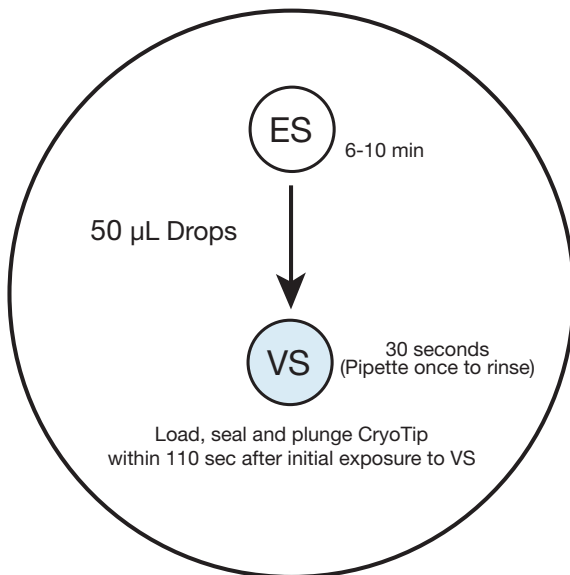
ALL PROCEDURES MUST BE PERFORMED AT ROOM TEMPERATURE (22–27°C)

 Prior to vitrification of blastocysts, refer to Collapsing Protocol, Human Blastocysts, FISl P/N 001882.

As an added precaution during the preparation procedure, carefully examine each CryoTip outside of the package. Prior to use, all CryoTip devices 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) 50 µL drop of ES.
2. Transfer embryo(s) (2 maximum), to the ES drop and expose undisturbed for 6-10 minutes.
 -  The specimen(s) will shrink and then gradually return to original size, indicating that equilibration is complete.
3. During above equilibration in ES, aseptically dispense one (1) 50 µL drop of VS 2 minutes prior to complete equilibration.
4. Transfer embryo(s) with minimal volume of medium from ES to the VS drop for 30 seconds before loading.
 -  To minimize floating, after 10 seconds pipette the specimen(s) to the bottom center of the VS drop.
5. Gently pipette embryo(s) once within VS drop to ensure complete rinse with VS.
 -  See reverse side for tips.
6. Load, seal and plunge CryoTip into LN₂ within 80 seconds, not to exceed 110 seconds after initial exposure to VS.
7. Refer to CryoTip Loading Protocol, FISl P/N 001430 diagram and product insert for detailed loading instructions.



KEY	
ES	Equilibration Solution
VS	Vitrification Solution
→	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.
- CryoTip devices should be pre-labeled with patient information, and assembled with connector and syringe (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 embryos (2PN to Blastocyst) 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.

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FUJIFILM IRVINE SCIENTIFIC – CORPORATE

1830 E Warner Avenue, Santa Ana, CA 92705 USA

Phone: 1 (949) 261-7800

Toll Free: 1 (800) 437-5706

Fax: 1 (949) 261-6522

Support: tmrequest@irvinesci.com