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Simplified Embryo Vitrification Protocol for HSV Device

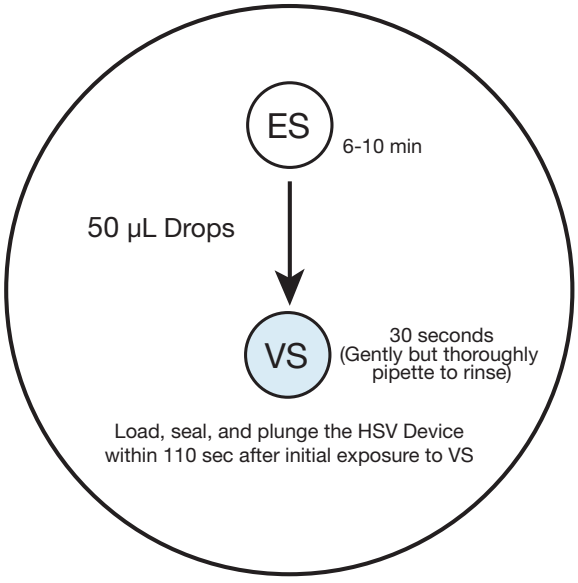
2PN to Blastocyst

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

Prior to vitrification of blastocysts, refer to Collapsing Protocol, Human Blastocysts, FISI P/N 002107.

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 drop to the VS drop for 30 seconds before loading.
5. Gently pipette embryo(s) once within VS drop to ensure complete rinse with VS.
   To minimize floating, after 10 seconds pipette the specimen(s) to the bottom center of the VS drop.
6. Load, seal, and plunge the HSV Device within 80 seconds, not to exceed 110 seconds after initial exposure to VS.
   Load after specimen is completely dehydrated and stable at the bottom of the VS drop.
7. Refer to HSV Device Loading Protocol, FISI P/N 002120 diagram and product insert for detailed loading instructions.
   See reverse side for tips.



KEY	
ES	Equilibration Solution
VS	Vitrification Solution
→	Transfer specimen to next drop

Simplified Embryo Vitrification Protocol for HSV Device

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.
- HSV Device should be pre-labeled with patient information, and the capillary tube should be connected with the longer end of the blue plastic insertion device, prior to starting procedure.
- Where possible, select only the best quality embryos (2PN to Blastocyst) for vitrification.
- The recommended HSV Device capacity is a MAXIMUM of 2 specimens.
- Process only as many specimen(s) as will be loaded per HSV Device 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 HSV Device within 80 seconds, not to exceed 110 seconds after initial exposure to VS.

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