EQUINE VITRIFICATION KIT

Store at 2º- 30ºC.

Contains: 1 x 8 ml vial of each:
VS1 - Vitrification Solution 1
VS2 - Vitrification Solution 2
VS3 - Vitrification Solution 3
Diluent

This Vitrification Kit is based on technology developed by the Animal Reproduction and Biotechnology Laboratory at Colorado State University, Ft. Collins, Colorado.

When using the enclosed material for long-term storage of vitrified equine embryos, it is essential to know the age and diameter of the embryos. It is highly recommended that embryos be 6 to 6.5 days of age (day 0 = day of ovulation), or approximately 8 days after administration of hCG. The ideal embryo stage for vitrification is a morula or early blastocyst.

Ideally, the outer zona of the embryos should be less than 300 microns in diameter and, in the case of blastocysts, the zona should be no less than ½ the normal thickness. It is highly recommended that an optical micrometer be used to measure the diameter of embryos to be vitrified. Embryos with an outer diameter of more than 300 microns will have a very low survival rate following vitrification.

Materials Needed:

1) 0.25 cc (1/4 mL) straws , 0.25 cc to 0.5 cc straw adapter plugs and 0.5 cc blue marking straws to be used for labeling.
2) 4 - Well Cluster Dish, Round Bottom, Sterile
3) Fixed volume 30 µl Embryo Handling Pipettor **(Necessary if *Alternative Procedure* is chosen. This procedure is highly recommended.)**
4) Liquid Nitrogen and container such as a Styrofoam box or Dewar
5) 10 mm canes, blue 10 mm goblets and blue cane tabs
6) Forceps (to support cane in liquid nitrogen)
7) Monoject syringe 1 cc withCook Syringe-Straw Connector 0.25 cc for drawing up media & embryos
8) Timer
9) Zoom Stereo Microscope
10) A 10/100 Eyepiece Micrometer and Wesco Stage Micrometer Scale
11) Semen thaw box with thermometer

Vitrification Procedure

Vitrification Apparatus: Set up apparatus in Diagram A and fill with nitrogen level 4 inches (10 cm) below the top before placing embryos in vitrification solutions. An aluminum cane, with attached 10 mm goblet, is secured with a pair of forceps in the liquid nitrogen, with 0.6 inch (12 mm) of the top of the goblet above the nitrogen level. (**Note** – no nitrogen in the goblet.)

Label the wells in a 4-well culture dish (round bottom wells recommended) with the following designations: **VS1, VS2, VS3 and Diluent**

1. Place 0.2 milliliter (mL) of the appropriate solutions in each well.

2. Move equine embryo from holding medium as follows:
VS1: 5 minutes VS2: 5 minutes VS3: 45 - 60 seconds

Note: VS3 step includes the time required to draw the embryo into the straw, seal the straw, and start the vitrification in liquid nitrogen vapor.

3. While the embryo is in VS2:
a) Draw approximately 90 microliters (µL) of Diluent (or a column 40 mm in length) into a straw.
b) Draw an air column 10 mm in length.

Note: These column lengths can be pre-marked on the straws with ink.

4. Immediately after moving the embryo into VS3:
a) Draw the embryo into the straw with approximately 30 µL of VS3 (a column approximately 14 mm in length)
b) Quickly draw another 10 µL air column and a second column of Diluent.

The second column of Diluent should be drawn up rapidly, until the PVC powder in the wick and powder end of the straw is saturated. A 0.25 - 0.5cc straw adapter can then be used to seal the straw. A 0.5 cc straw that has been previously labeled with information appropriate for the embryo can be attached to the 0.5 cc end of the adapter (see Diagram C - Media Compartment Lengths).

(Continued on Reverse Side)

Two of the most common mistakes in Vitrification:

1) Pipetting too much fluid when moving the embryo from VS1 to VS2 and VS2 to VS3
2) Leaving the embryo in VS3 for more than 45-60 seconds.

Alternative pipetting procedure:

Instead of using the straw to move the embryo between the drops we suggest a pipettor that delivers only 10-30 microliters of fluid.

We also suggest that the diluent drops of 90 microliters are made with a pipettor that delivers the exact 90 microliters so the entire drop can be loaded. This seems to save time and assures the correct volume is loaded.

NOTE: Alternative Procedure:
Some practitioners may prefer the following alternative method of exposing the embryo to VS3 and loading the straw. While the embryo is in VS2, deposit a 30 microliter drop of VS3 in an empty dish or in the lid of a dish. Move the embryo from VS2 into the drop of VS3, expelling as little medium as possible, preferably less than 2 microliters. Immediately draw up the embryo and VS3 by holding the straw vertically over the microdrop (see Diagram B on reverse side).

Vitrification Procedure continued

5. **Within 45-60 seconds after the embryo is placed in VS3, the straw should be sealed and placed in a plastic straw goblet that has been previously immersed part way (within 8-12 mm of goblet top) in liquid nitrogen and is already cold (see Diagram A).**

6. **Leave the straw in the vapor in the goblet for 1 minute. Then plunge cane (with goblet) into the liquid nitrogen, so that goblet fills completely with liquid nitrogen.**

7. **Transfer cane and goblet (containing the straw) to a liquid nitrogen holding tank for storage.**

Thawing

1. **Remove the straw from the liquid nitrogen and hold in room temperature air for 5 - 10 seconds, then place into 35ºC (95ºF) water for 20 seconds.**

2. **Holding the labeled end of the straw, mix the contents of the straw by flicking the straw (similar to a thermometer) 5 to 7 times.**

3. **Lay the straw on a horizontal surface for 6 to 8 minutes at a temperature of 20º- 25ºC, prior to loading it into a transfer gun for transfer into a recipient mare.**

As an alternative, some practitioners prefer to remove the embryo from the straw and let it sit in Holding medium for a few minutes prior to transfer. Typically the embryo rehydrates in Holding media during this short incubation time. The embryo can then be reloaded into a straw and transferred.

Diagram A

Important: Set up apparatus in Diagram A and fill with nitrogen just below the top of the plastic goblet before placing embryos in vitrification solutions.

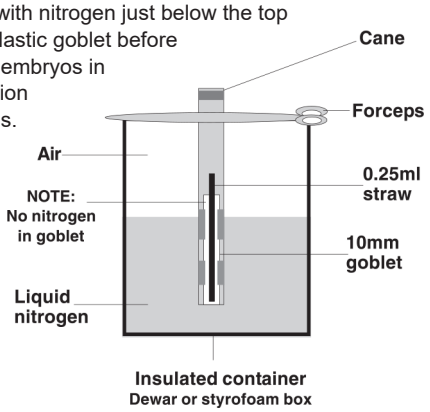
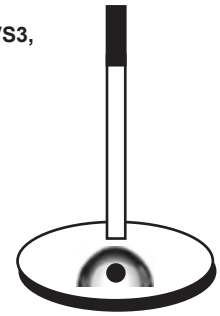


Diagram B

Exposure of embryo to 30 µL microdrop of VS3, which is then drawn directly into straw.

30 µL drop of VS3 in empty petri dish

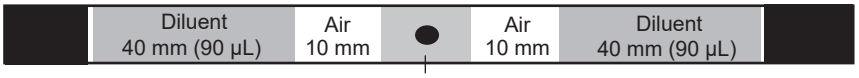
Embryo in VS3



Straw held vertically in preparation for aspirating embryo and VS3 microdrop

Diagram C

Media compartments in 115 mm straw and column lengths (not to scale).



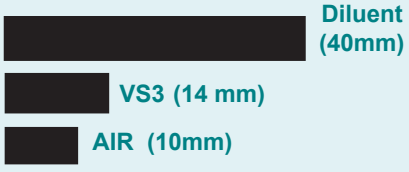
Plug + Label donor, date, sire information

VS3 + embryo 14 mm (30 µL)

Cotton Plug

Gauge for Column Lengths

(to scale)



Manufactured by:

Pullman, WA 99163 USA
509.595.3568