



Vitrification



Preparation

Ensure all media are warmed up to 37 °C and mixed well before use.

We would strongly advise you to read through all the steps of the vitrification/thawing procedure before starting the procedure.

Preliminary steps

1. In a 4-well dish fill the first well with 300 µl of VitriStore Pre-vitrification Medium, the second with VitriStore Freeze 1 and the third with Vitri-Store Freeze 2 solution.
2. Make sure that the liquid nitrogen is available to ensure fast work flow.
3. Next open as many packs of vitrification devices as will be required for the vitrification step. Place the separate parts of the vitrification device on the workbench for easy access later in the procedure.

Freezing preparation

1. Transfer the embryos from the blastocyst cell culture medium into the first well (PVM).
2. Process the embryos according to the schemes by transferring them from one well to the next.

Vitrification

1. Using an attenuated pipette or an equally suitable device, place a maximum of 2 blastocysts in a volume of approximately 0.3 µl of VitriStore Freeze 2 on the tip of your vitrification straw.
2. Place the vitrification straw in the outer sheath and seal it as indicated in the instructions for use of the vitrification device.
3. Plunge the sealed device into the liquid nitrogen.

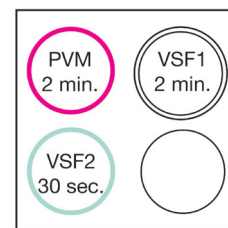
Morulae

Early blastocysts

Blastocyst after artificial shrinking

Solutions and application at room temperature

Well 1	Well 2	Well 3
PVM	VSF 1	VSF 2
2 min.	2 min.	30 sec.

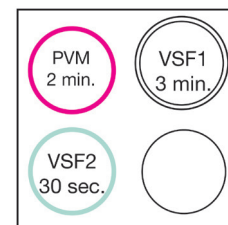


Blastocysts

Expanded blastocysts

Solutions and application at 37 °C

Well 1	Well 2	Well 3
PVM	VSF 1	VSF 2
2 min.	3 min.	30 sec.



* Before starting the vitrification procedure, in order to reduce the negative effect of the blastocoel, expanded blastocysts should be collapsed by reducing the volume of the blastocoel artificially with a glass pipette. (Vanderzwalmen et. al., 2002; Sonet et. al., 2003; Hiraoka, 2004)

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