

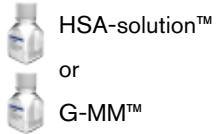
CRYO PRESERVATION

Blastocyst stage

Directions for supplementation of un-supplemented G-Series™ media can be found in the G-Series Manual on www.vitrolife.com. Once supplemented, the media should be used as the G-Series PLUS media described below.

Day 5 or 6

Freezing:



1. Supplement the solutions, 1.0 mL of albumin + 9.0 mL of freeze solutions.

all steps ambient temp and atmosphere

2. Prepare a 5-well dish with BIM™, BFS1™ and BFS2™.



3. Rinse blastocysts for freezing in BIM.



4. Place the blastocysts in BFS 1 for



10 min

5. Move the blastocysts to BFS 2 for



7 min

6. During these 7 min rinse the freezing straw with BFS 2 in a separate dish/well.

7. After 7 min, transfer the blastocysts to a new well with BFS 2 for loading into straws.



8. Load the blastocysts into the straws and seal. **Do not exceed 15 min in BFS 2.**



For detailed freezing program, see G-Series Manual.

9. Immediately place into freezing chamber at

-6°C

10. Wait for

2 min

11. Manually seed the straws with liquid nitrogen (LN₂) cooled forceps close to the cotton plug at

-6°C

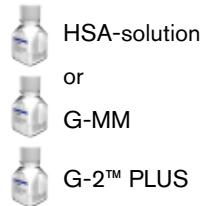
12. Hold the temperature for

2 min

13. Continue the freezing program until end.

14. Plunge into LN₂.

Thawing:



1. Supplement the solutions, 1.0 mL of albumin + 9.0 mL of freeze solutions.

all steps ambient temp and atmosphere

2. Prepare a 5-well dish with BTS1, BTS2, BTS3 and BIM



3. Remove the straw from LN₂ and air thaw for

30 sec

4. Place straw in water bath

30°C 30 sec

5. Remove and carefully wipe.

6. Cut the straws with sterile scissors and expel the blastocysts into BTS 1™.



7. Move the blastocysts to BTS 2™ immediately and leave for



5 min

8. Transfer the blastocysts to BTS 3™ and leave for



5 min

9. Transfer to BIM™ and leave for



5 min

10. Transfer the blastocysts to a second well of BIM on a stage warmer at



37°C 5 min

11. Wash the blastocysts once in G-2 PLUS and place into culture until transfer at



37°C 6 % CO₂

Embryo transfer is performed according to the Embryo transfer protocol