

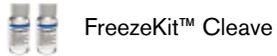
CRYO PRESERVATION

Cleavage stage embryos

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 2 or 3

Freezing:



1. Prepare dishes with 0.5-1.0 ml each of Equilibration solution (ES) and Freezing solution (FS) at

**ambient temp
and atmosphere**

One dish per patient and solution



2. Place the embryos in ES and rinse properly. The embryos can stay in ES for 10 minutes.

3. Transfer the embryos into the FS and load the embryos into straws immediately. Total exposure time in FS is

10 min

Pre-rinse all utensils, including dishes and pipettes when applicable with G-RINSE™

4. Seal the straws and place into the freezing machine at room temperature and commence the freezing program, see G-Series manual.



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

-6°C



6. Continue the freezing program.

7. Plunge the straw into liquid nitrogen when the freezing program has ended and arrange for storage at

-196°C

Thawing:



1. Prepare dishes with 0.5-1.0 ml of TS1, TS2 and ES into respective dishes at

**ambient temp
and atmosphere**

One dish per patient and solution



2. Remove the straw from liquid nitrogen and expose to air

30 sec

Place the straw in a water bath at

30°C 45 sec

3. Remove the straw and wipe it carefully.

4. Open the straw using aseptic technique and gently expel the embryos into TS1 and leave at

ambient temp 5 min

5. Move the embryos into TS2 and expose for

ambient temp 5 min

6. Move the embryos into ES and expose for

**ambient temp
and atmosphere 5 min**

7. Rinse embryos properly in G-1 PLUS™ or G-2 PLUS™ depending on developmental stage and culture until transfer at

37°C 6 % CO₂

For embryo transfer, see separate Embryo transfer protocol