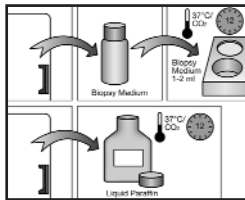


Procedure (Embryo biopsy):

Day 0 to 2

1. Follow recommended protocol for fertilisation and cleavage stage culture in either BlastAssist® System (Cat. No.1039/1040) or ISM1™ (Cat. No. 1050/1150)

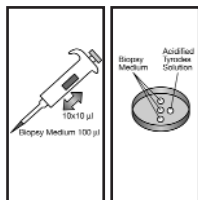


Day 2 (2 days after egg collection)

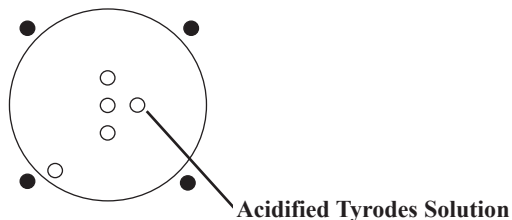
2. In a tissue culture dish place a sufficient volume of Biopsy Medium for the biopsy of the embryos, overlay with pre-equilibrated Liquid Paraffin (Cat.No. 1010) and warm to 37°C in a CO₂ environment for a minimum of 2 hours (allow 30 µl per embryo plus 100 µl for flushing the pipette tip as necessary). Remember to pre-equilibrate sufficient Liquid Paraffin for the biopsy procedure (allow 4 ml per embryo).

Day 3 (day of embryo biopsy)

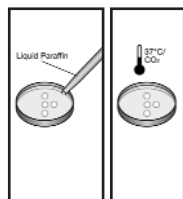
Cleavage stage embryo biopsy is carried out early in the morning of Day 3 post-insemination.



3. Half an hour before the biopsy, prepare a biopsy dish for each embryo and label it with the patient's name and embryo number. Take an automatic pipette set at 10 µl with a sterile tip and flush the tip (x10) with Biopsy Medium. Pipette three droplets of Biopsy Medium and one droplet of Acidified Tyrodes Solution (Cat. No. 1060) as shown in the diagram.



Immediately cover the dish with 4 ml of pre-equilibrated Liquid Paraffin to avoid evaporation and store the dishes at 37°C in a CO₂ environment until required. At the same time prepare additional microdrops or wells/dishes of extended culture media correspondingly labelled, for wash and final culture of the embryos whilst the diagnosis is carried out.

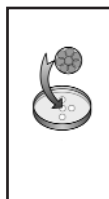


Check list

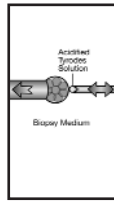
1x biopsy dish per embryo

1x dish per embryo for washing.

Appropriate number of microdrops or wells/dishes for final culture of the biopsied embryos.

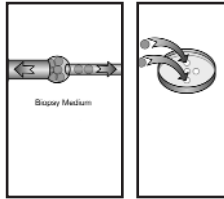


4. Take the appropriate pre-equilibrated and labelled biopsy dish and aseptically transfer the embryo into the middle droplet of the dish.

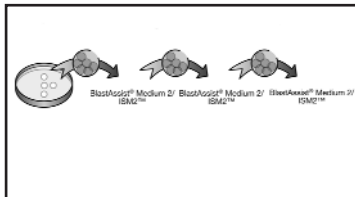


5. Prime pipettes with the appropriate solutions and immobilise the embryo to be biopsied. De-compaction should be completed within a short period (1-10 minutes) and the embryo should be observed throughout the process.

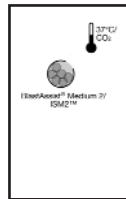
6. Bring the Acidified Tyrodes Solution pipette into contact with the zona of the embryo. Take care only to use the smallest possible volume of Acidified Tyrodes Solution to facilitate the drilling process. See the section “Zona drilling.”



7. Once a small hole in the zona has been obtained, the blastomeres should be readily accessible with the sampling pipette. To minimise the reduction in mass, one or two of the smallest blastomere(s) are biopsied and placed in the corresponding droplets of Biopsy Medium.



8. At the end of the biopsy, the embryo should be transferred to the separate dish for washing, where it is transferred through microdrops or wells of extended culture medium (BlastAssist® Medium 2 or ISM2™) to remove all traces of Biopsy Medium and Acidified Tyrodes Solution.



9. Finally transfer the embryo into a new dish with fresh pre-equilibrated microdrops/wells of extended culture medium overlaid with pre-equilibrated Liquid Paraffin.

10. The biopsy dish with the isolated blastomeres is then ready for sample preparation.

When the PGD is complete, assess the morphology of each of the embryos and count the number of cells as accurately as possible to get an indication of division post biopsy. The embryo transfer is usually performed on Day 5, after the genetic diagnosis has been completed.

In consultation with the other members of the PGD team and finally with the couple themselves, select a maximum of two unaffected embryos with the best morphology for transfer.