Preparation: EmbryoSlide+ Culture Dishes

The EmbryoSlide+ culture dish is designed specifically for culture of embryos in the EmbryoScope+ time-lapse incubator. The culture dishes hold up to 16 embryos each, and are made of polystyrene certified for use in IVF procedures. They are delivered as individually packed, sterile dishes in convenient handling pouches. Double handling fins provide stable handling, and the barcode label ensures correct registration of patient information and improves the workflow.

Vitrolife recommends preparing the EmbryoSlide+ dishes on the day before use. Prepare the dishes on a non-heated surface to avoid evaporation. The procedure described below requires less than two minutes per culture dish.

The EmbryoSlide+ culture dish

Embryos are cultured in individual microwells. The microwells are placed in two separate culture compartments, each comprising eight wells cultured under a common medium droplet. The complete culture area is covered by a common oil layer.

Each well carries a number from 1-16 for identification under a stereo microscope. Each well number corresponds to the well identification number in the EmbryoViewer software.

Four large rinsing wells are available outside the culture compartments. These special wells can be used during embryo handling (identified as A-D).

Each batch of EmbryoSlide+ culture dishes must pass our stringent MEA testing procedure before being released for sale. This is part of the Vitrolife quality assurance.

EmbryoSlide+ preparation

Prepare the EmbryoSlide+ culture dishes on the day before use. Prepare one dish at a time to minimise the handling time of each dish.

The EmbryoSlide+ culture dishes should be prepared with cold medium and oil on a non-heated workbench to avoid evaporation of medium during preparation.

When they have been prepared, the culture dishes must equilibrate overnight before loading embryos into the microwells.

Use a stereo microscope to control the process.

The recommended procedure for preparing the culture dishes is outlined on the next page.
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<th>Step</th>
<th>Action</th>
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<td></td>
<td><strong>Remove the culture dish from the pouch.</strong> Prepare the dishes with cold culture medium and oil on a non-heated workbench to avoid evaporation. Prepare one dish at a time to minimise the handling time of each dish.</td>
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|      | **Fill all microwells with culture medium**<sup>*</sup>  
*Use a micropipette tip with a max. diameter of 200μm*  
For most tips, one filling of the micropipette tip will suffice to completely fill eight microwells. Let the tip touch the side of the microwells during the procedure. This will help prevent bubble formation. Slightly overfill the microwell to create a convex meniscus. |
|      | **Fill the wells and two culture reservoirs. Load a total of 180μL culture medium**<sup>*</sup> into each reservoir. *Use a standard pipette.*  
Slide the tip over the wells while releasing the medium to avoid bubble formation. Make sure that the pipette tip touches the microwell droplet in each well. Fill the reservoir completely, including the pipetting zone. |
|      | **Fill each rinsing well with max. 30 μL and min. 25 μL of culture medium**<sup>*</sup> |
|      | **Immediately load 1.6 mL of culture oil**<sup>*</sup> into the reservoir  
It is important to apply the oil overlay quickly to avoid evaporation of medium. Make sure that all wells, including the rinsing wells, are covered with a confluent oil layer to eliminate evaporation of medium. |
|      | **Push up larger bubbles with a micropipette and remove them** |
|      | **Cover with the lid and equilibrate overnight.**  
Identify and remove any bubbles under a stereo microscope  
Attach the barcode label to the dedicated labelling area on the dish. |
|      | **Load embryos into the center of the microwells.** |
|      | **Place the dish in the EmbryoScope+ incubator.**  
*If you want to change medium during the culture period:*  
From each culture reservoir remove 90μl old medium and add 180μl new warm equilibrated medium. It is important to remove and add the medium in a constant flow and keep the tip of the pipette away from the embryos. |

<sup>*</sup>Vitrolife recommends using G-TL medium, designed specifically for continuous culture with time-lapse technology and OVOIL™ 100% paraffin culture oil for complete control of your culture system. Vitrolife products are produced under highly controlled processes.