

# Preparation of EmbryoSlide Culture dishes

The EmbryoSlide® culture dish is specifically designed for the individual culture of up to 12 embryos in the EmbryoScope™ time-lapse incubator. The dish also contains wells designed for rinsing.

The EmbryoSlide culture dishes are designed for easy and stable handling and are made of culture-tested polystyrene. They are delivered in sterile, single pouches.

Vitrolife recommends preparation of EmbryoSlide culture dishes a day before use. Prepare the dishes with cold medium and on a non-heated surface to avoid evaporation. The procedure described below requires less than 1.5 minutes per dish.

## General characteristics of the EmbryoSlide culture dish

The embryos are incubated in individual microwells in a small (25 µl) volume of culture medium under a confluent oil cover.

Each well carries a number from 1 to 12 for identification under a stereo microscope. Each well number corresponds to the well identification number in the EmbryoViewer® software.

Two rinsing wells are available at each end of the dish. These special wells can be used during embryo handling (identified as A-D).

There is a slight variation in how much the temperature decreases in the microwells (approx. 0.6°C) and the rinsing wells (approx. 0.7°C). Both measurements have been performed on a 37°C heating plate over a period of two minutes. This represents normal dish handling time.

Each batch of EmbryoSlide+ culture dishes must pass our

stringent MEA testing procedure before being released for sale as part of the Vitrolife quality assurance.

## Preparation for use on the next day

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 the 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.

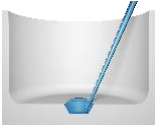


Step

Action



**Remove the culture dish from the pouch.**  
**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.**



**Fill all microwells with a small amount of culture medium\***  
**Use a micropipette.**  
 Slightly overfill the microwell to create a convex meniscus.



**Immediately fill all needed wells, including the rinsing wells, with 25  $\mu$ L of culture medium\*. Use a standard pipette.**



**Immediately load 1.4 mL of culture oil\* 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. Add an additional 25 $\mu$ L of culture oil per well not filled with medium.

**Push up larger bubbles with a micropipette and remove them**  
**Cover with the lid and equilibrate overnight.**  
**Remove any bubbles that may have formed.**



**Load embryos into the center of microwells. Use a micropipette.**



**Place the dish in the EmbryoScope incubator.**

**If you want to change medium during the culture period:**  
 From each culture well remove 20  $\mu$ L old medium and add 20  $\mu$ L 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.

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

Refer to the TECHNOTE *Additional notes on EmbryoSlide® culture dish preparation* for further recommendations about optimal handling of the EmbryoSlide culture dish.