Using the pH validation dish in EmbryoScope+

Validation of culture media pH is an important aspect of quality assurance in the IVF laboratory. The optimal pH for culture of human embryos is between 7.2 and 7.4. For long term incubation of human cleavage stage embryos most IVF laboratories use a culture medium that is based on a bicarbonate buffer system and requires a controlled atmosphere with elevated carbon dioxide (5 – 8%).

The pH Validation Dish is comprised of an EmbryoScope+ slide with a non-embryotoxic gas permeable silicone lid which forms a watertight seal. The dish is designed to allow pH equilibration of a bicarbonate buffer system while limiting evaporation in a dry incubation environment without the use of an oil overlay. The dish is specifically designed for use in the EmbryoScope+ and is not to be used for embryo culture as it is not sterilized and is only suitable for equilibration from 8-24 hours. The dish is only suitable for measurements using a blood gas analyser of a micro or mini-pH probe (maximum immersion depth is 5-7 mm).

The pH validation dish

The pH validation dish comes individually packaged with a MEA tested label attached.

The format of the dish is exactly the same as the EmbryoSlide+ dish and therefore the dish is particularly well suited for pH validation in the EmbryoScope+ incubator.

To use the dish for pH validation of culture media in your EmbryoScope+ incubator, use the option ”insert equilibration dish” when inserting the dish into the slide holder.

The pH validation dish is non-embryotoxic and has a lid designed to allow pH equilibration of bicarbonate buffered media while limiting evaporation.

How to use the pH Validation Dish

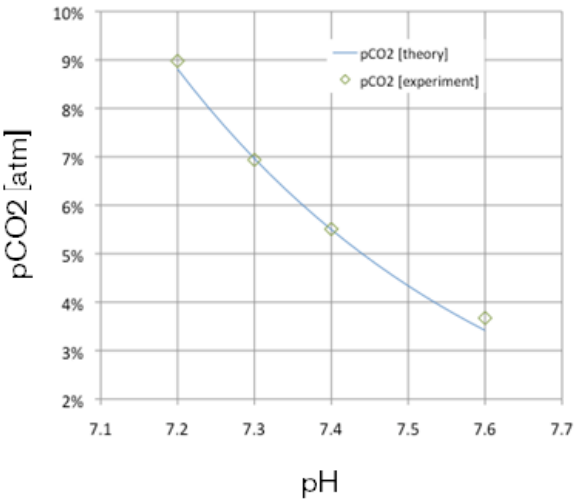
1. **Remove the dish from the pouch and remove the silicone lid.**
2. **Fill the dish with 3.5 mL of culture medium.**
3. **Place the silicone lid firmly on the validation dish. Press the lid firmly down along the edge and ensure a smooth surface.**
4. **Insert the dish into an incubator with appropriate gas and temperature conditions. Leave it there for 8 to 24 hours.**
5. **Remove the dish from the incubator and immediately turn it briefly upside down.**
   - Turning the dish briefly allows condensation droplets to re-equilibrate with remaining media for correct pH measurement.
6. **Remove the lid from the dish and immediately measure pH directly in the dish.**
   - Measure pH by submerging a micro pH electrode fully into the medium sample. Both sensing surface and reference junction must be fully submerged.
   - Alternatively, withdraw a medium sample (preferably less than 0.5mL) for measuring pH with a blood gas analyzer. Take care that medium sample is not exposed to degassing.

Do not transfer the medium sample to another tube as this will cause degassing from the sample resulting in pH measurements that do not reflect the culture conditions.

Notes about the pH, CO2 and dependence on environmental fluctations

Carbon dioxide and pH of IVF culture medium

The pH of the medium is controlled by the carbon dioxide concentration in the incubation chamber. The figure below shows the theoretical relationship between CO_2 concentration in an incubator (in % atm) and the resulting equilibrium pH as a continuous blue curve. The green points are experimental values from *D. Gardner and M. Lane (2000) Embryo culture systems in Handbook of In Vitro Fertilization, 2'nd Ed, CRC Press p. 232-234.*



Calculation based on a medium with a total alkalinity of 27.4 mmol/kg at 37°C, CO2SYS, van Heuven et al 2009: http://cdiac.ornl.gov/ftp/co2sys/CO2SYS_calc_MATLAB/

Carbon dioxide in the incubator is in equilibrium with dissolved carbon dioxide, carbonic acid, bicarbonate and carbonate:

$$CO_2(g) \rightleftharpoons CO_2(aq) \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

The pH of the media at physiological pH can be approximated by the Henderson-Hasselbach equation:

$$pH = pK_a + \log_{10}([HCO_3^-]/[CO_2])$$

The relationship between carbon dioxide concentration within an incubator and the resulting pH in a given medium shows a remarkable resilience to pH change even after substantial change in CO_2 concentration. Large changes in CO_2 thus only cause minor changes in pH. We recommend a stable CO_2 measuring device that gives accurate results in the desired range of 5-8%.

Note

When measuring and interpreting media pH it is important to remember that:

1. The pH-CO_2 relationship is altitude dependent. That means that at altitudes higher than sea level, CO_2 % must be higher in order to obtain the same pH.
2. Molecular diffusion through the medium layer to reach a stable equilibrium takes several hours. It is thus essential that the medium sample is properly equilibrated before the pH can be reliably measured.
3. When handling small media samples care must be taken to avoid temperature changes (see figure to the right) and CO_2 diffusion into the syringe or other handling device. The figure (right) shows the moderate effect of reducing media temperature to room temperature while maintaining 5% CO_2. Cooling the media to RT will decrease pH by 0.07 which must be corrected for.
4. We do not recommend transferring the medium sample to another container before measuring the pH value as this transfer will likely lead to changes in pH due to degassing of the medium sample.