CultureCoin Preparation for Miri® Time-Lapse Incubator

Introduction

The CultureCoin is a proprietary device designed with fourteen (14) individual culture wells for embryo culture.

The right procedure of preparation of the CultureCoin is essential to avoid bubble formation. It is recommended to pre-incubate the CultureCoin inside a humid incubator prior to using it for embryo incubation.

The CultureCoin Design

The CultureCoin has 14 individual culture wells numbered from 1 to 14. Each well has two additional wells for embryo washing/ collection.

The CultureCoin also has a pH reservoir for measuring pH. The diameter of the dish is 7 cm.

The CultureCoin is made of BASF 158 polystyrene. It has EC certificate with the external testing done at Embryotools in Barcelona, Spain.

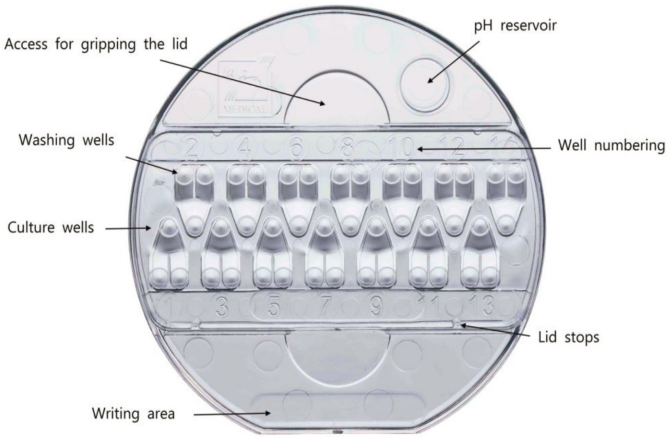

Figure 1. *The CultureCoin design with its detailed description.*

CultureCoin Preparation Timeline

Preparation of the CultureCoin, media and oil should be done a day before loading embryos.

We have simplified the process into three simple steps as seen in Figure 2.

STEP 1: Pre-Equilibration Measures (Day 0 or Day Before Use)

Culture Media, Oil and CultureCoin Pre-equilibration

Morning Procedure:

a. Equilibrate the media according to your protocol e.g. in a clean test tube and place it in a conventional humidified incubator for 4 to 6 hours.

b. Pre-equilibrate overlay oil in a conventional humidified incubator for 1—2 days prior to use.

c. Leave the CultureCoin with the lid, inside a humidified incubator for 4 to 6 hours.

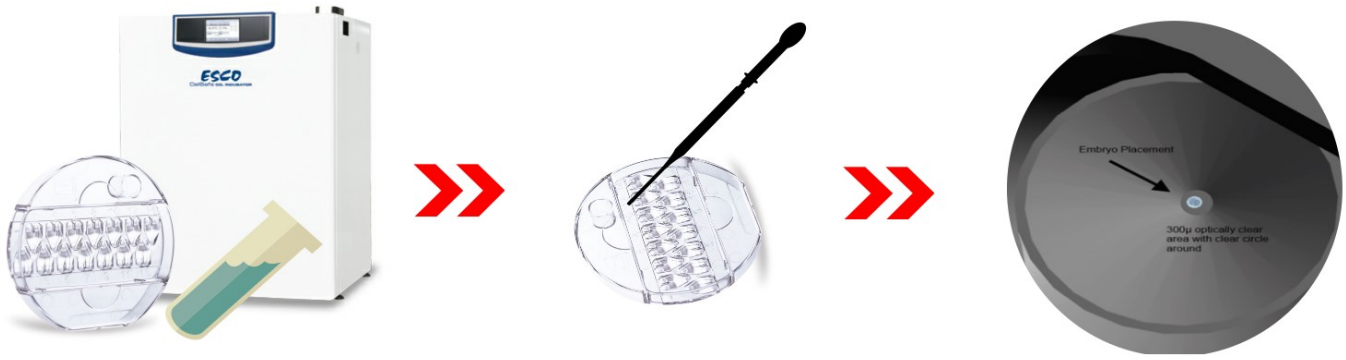

Figure 2. *CultureCoin Preparation Timeline in three easy steps.*

STEP 2: CultureCoin Loading (Day 0 or Day Before Use)

Afternoon Procedure:

a. Load the CultureCoin with media and oil inside an IVF workstation.

b. Fill the pre-equilibrated media from the wall of the well, as shown in Figure 3 using a 200 µL dispensing pipet. Inject 25 µl for each well. *Note: Filling the washing well is optional, they each hold 23 µl .*

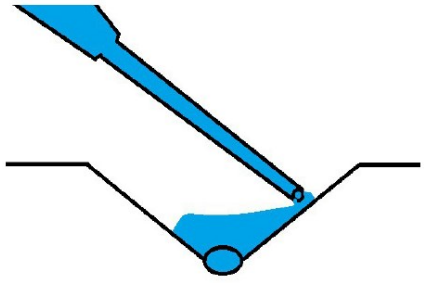

Figure 3. *Do not inject the media directly on the bottom of the well. Fill it on its wall.*

c. Remove any visible bubbles from the media.

d. Cover the media with 3 - 3.5 ml of pre-heated oil.

e. Put the CultureCoin with the lid inside an incubator and leave it for > 12 hours for equilibration.

STEP 3: Bubble Removal and Oocyte/ Embryo Loading (Day 1 or Day of Use)

a. Check for air bubbles in the culture wells. Remove any bubbles with a stripper tip.
Note. Larger bubbles that are suspended between the media and oil may successfully be removed by touching the bubbles with a media filled stripper tip, which will then float up into the stripper tip.

b. Load the embryos into the micro wells with a plastic stripper tip, The diameter of the stripper tip will vary depending on the day of embryo development — for oocytes and embryos up to Day 4 transfers, a 120 to 130 µm diameter should suffice; for later stages or blastocysts, use a 250 µm stripper tip.
Notes:
(1) try to place the embryos in the center of the micro well as this will give better videos and make the automatic well-finding much easier.
(2) It is recommended to use a plastic stripper tip as to not scratch the wells as it normally occurs with the use of glass tips. Having scratches in the wells will hinder subsequent photos and videos.

c. *Optional:* fill the pH reservoir and insert the silicone plug.

d. Securely place the CultureCoin in the insert located on the baseplate of the selected chamber. *Note: Flat-side of the CultureCoin should be facing you in order to ensure proper positioning.*

e. Once ready, start Time-Lapse.

STEP 4: Changing the media after Day 3 embryo Culture

This step is only applicable for culturing process that involves sequential media. This step should be done as quickly as possible to prevent fluctuations in temperature and pH.

Note: Culture media should be pre-equilibrated before commencing this step.

a. Use a stripper pipette to quickly and simply move the embryos into their corresponding wash wells.
b. Use a 200 µL dispensing pipette to aspirate the old media. The plastic tip has to be slid slowly down the wall of the well, along the side of the well and under the oil cover. Aspirate the old medium from each well and dispense the collected media into the pH reservoir of the CultureCoin. Ensure to leave behind a small amount of medium as to not completely dry it up.

   Note: The use of a larger volume tip allows you to quickly aspirate most of the old media from all the wells in a single pass rather than going back and forth (aspirate/ expel) multiple times in order to remove media from all wells.
c. Quickly fill the tip with fresh pre-equilibrated media and start aliquoting into the wells in the exact technique outline in step 4.b. Check for and remove any air bubbles that may have been introduced in the process, as described in Step 3a, and that all wells are still adequately covered with oil overlay.

d. Place the CultureCoin back in Miri TL, with the embryos still in wash wells. Allow the refreshed wells at least 30 minutes to re-equilibrate. Then remove dish, quickly move embryos from wash wells to corresponding culture well.

   Note: Doing this step minimizes temperature and pH fluctuations.

e. Check for placement of the embryos and then quickly load the CultureCoin back into the Mir TL.

f. Resume Time-Lapse.