Egg Warming Protocol

Egg Warming with Kitazato kit, Oosafe 6-well dish, and Cryotop/Cryolock straws

LUCINA Egg Bank

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- Form LEB - 001
- Revision Ver 1.4
- Effective 05-28-2024

Egg Warming Procedure

Materials/Supplies

Kitazato warming (thawing) media kit

- Thawing Solution (TS): 2 X 4ml vial
- Diluent Solution (DS): 1 X 4ml vial
- Washing Solution (WS): 1 X 4ml vial

Note: The above media kit is good for thawing up to 4 straws.

Oosafe 6-well dishes

Petri Dishes (35mm, Falcon 351008 or equivalent)

Cooling Rack (Ref. 84010 or equivalent): Blue Styrofoam box for liquid nitrogen

Stripper tips (170-200µ)

Heated dual stage with Stereomicroscopes

Stopwatch or Timer (with count up function is ideal)

Liquid Nitrogen

Tweezers

Micro pipette and tip: 100-1000µL

Egg/embryo culture media with 10 and 20% protein

Preparation for Oocyte Warming and Space Warming (Thawing)

01 Warm TS vial (sealed) with a Petri Dish in an incubator or warm chamber to 37-38°C (>1.5 hours).

02 Take out DS and WS from the refrigerator to warm at room temperature roughly about 25° C. (>1 hour)

03 Retrieve the cane which has the specific Cryotop or Cryolock, quickly immerse the cane in a Cooling Rack filled with fresh liquid nitrogen.

04 Write **DS, WS1** and **WS2** on the lid of a 6-well dish. Gently invert each vial of DS and WS twice to mix contents. **Pipet 300µL** each for **DS, WS1** and **WS2** into the 6 well dish with a micropipette. Place it on the microscope stage and replace the lid.

05 Remove TS vial and the Petri Dish from the incubator and place the Petri Dish on the microscope stage. Gently invert the vial of
TS twice to mix the media and pour the full contents into the Petri Dish.

- Pour the entire vial **(4ml)** of TS medium into the petri dish and keep it in the warm chamber with humidity until you start egg thawing. **(See further instructions on the last page).**
- A petri dish with **4 ml** of TS media is good for two times of thawing.

Warming (Thawing) Procedure

Step- 1

Carefully twist and remove the straw cap from the Cryotop or Cryolock while submerged in liquid nitrogen. Prop it against the corner of the Cooling Rack.

Step- 2

Be ready to use stripper tip(s). Set up the stopwatch. Check the time with the stopwatch for the following steps.

Step- 3

Quickly immerse Cryotop or Cryolock into TS on the microscope stage. It should be within 1 second (Don't do this too quickly. It will create air bubbles). Find the Oocyte(s) adjusting the focus on the black mark area of the straw tip. The oocyte(s) will come off from the straw, otherwise aspirate it after blowing a small amount of TS medium on the oocytes. One minute after immersing into TS, gently aspirate the Oocyte(s) with the stripper tip and move to DS drop.

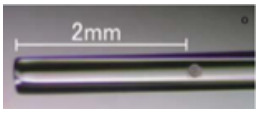
- Don't carry over big volume from TS to DS. Aspirate only 2mm from the end of the tip.

Step- 4

Blow out only **TS** into the **BOTTOM** center of **DS** slowly, then gently place the Oocyte(s) on the bottom of the TS layer. Leave it for 3 minutes. This is for a gradual transition from **TS** to **DS**.

Step- 5

After 3 minutes of immersing into **DS,** use a stripper tip to gently aspirate the Oocyte(s) from the **DS.** Aspirate the oocytes along with **DS medium** until the Oocyte(s) reaches 2mm from the tip of the stripper tip.



Step- 6

Blow out only DS into the BOTTOM center of WS1 slowly, then gently place the Oocyte(s) on the bottom of the well. Leave it for 5 minutes.

Step- 7

5 minutes after immersing into **WS1**, aspirate the Oocyte(s) with minimal volume of **WS1** with the stripper tip and transfer it to the **TOP** center of **WS2**. After the Oocyte(s) free-falls to the bottom of the **WS2**, repeat the process of placing the oocyte(s) on top of the **WS2** and allowing them to free-fall.

- You can perform the **WS2** wash step at **37°C** heated stage.

Step- 8

Transfer the oocyte(s) to a culture dish containing culture medium with 20% SPS. Incubate the oocyte(s) in a 37°C incubator to complete recovery. **ICSI can be performed in 2-3 hours.**

Additional instructions: Please do not deviate from this protocol

Temperature of TS Medium

- Temperature of TS medium at the time of egg thawing is very important. Keep TS media in a warm chamber with temperature 37-38°C until thawing the eggs.

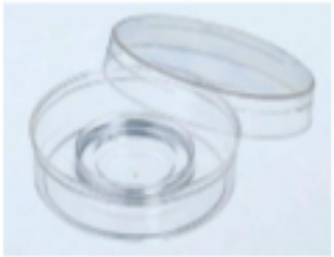
- Pour the entire amount (4ml) into the 35mm falcon petri dish right before egg thawing.

- If there is any delay, you can store the 35mm falcon petri dish containing TS solution in the 37-38°C warm chamber or incubator without CO2 gas until thawing the eggs.

Dishes for TS Step

- Dish for egg thawing (TS step): We recommend to use Petri dish (35mm, 4ml TS solution). You can use the same TS solution up to 2 times. After using the TS solution once, please keep the petri dish in a 37-38°C warm chamber with humidity for 10-20 minutes before reusing it.

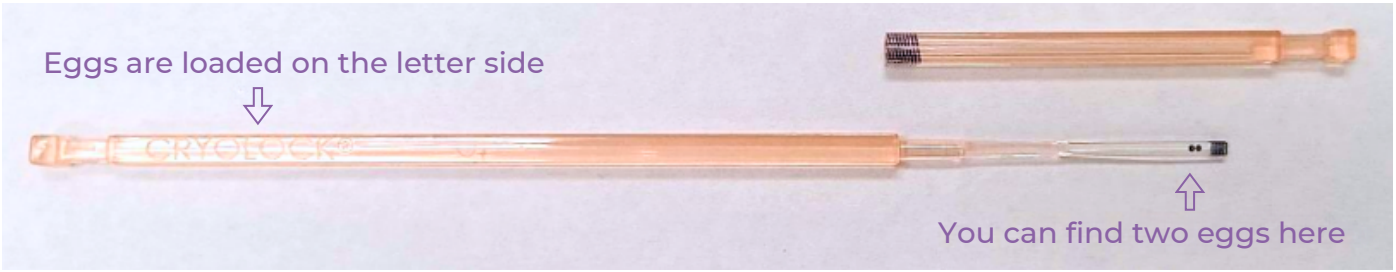
- Some embryologists like to use Reprolife warm plate or Inner-well dish for TS steps (see the pictures below). If you want to use those dishes, we recommend warming the dishes with 2 ml of TS medium in the warm chamber with humidity at least for 1 hour. Use only once and discard it.



Cryotop straw: Finding the eggs

- The frozen eggs are located on the same side of ‘Identification marks’.

Cryolock straw: Finding the eggs



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