

# SPB1 (0.3M sucrose in PB1)

Cat. No. R-S187

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## Usage

Usable for thawing rat cryopreserved embryos.

## Storage

Keep them in 4°C until use. Use all the media once opened and avoid using the remaining because of the quality degradation.

## Preparation

1. Warm up SPB1 at 37C before use.  
[Don't open the vial when you warm up SPB1 in CO2 incubator, to avoid the change of its pH.]
2. Make 3 drops of 100 uL mR1ECM on petri dish, for each frozen tube containing embryos.  
Put the dropped petri dish into CO2 incubator for more than 30 min.  
[Use HTF instead of mR1ECM, if you handle the pronuclear stage embryo. Similar to the following.]

## Thawing

1. Pick up the frozen tube containing embryos from LN2 tank, and immediately take away its cap and remove LN2 in tube, and subsequently keep the tube at room temperature for 30 sec.
2. Add 0.9 mL of 37C-warmed SPB1 into the tube, conduct the rapid and gentle pipetting within it, and transfer the solution onto petri dish.  
[Speedy handling is needed in above procedure, because the thawed cryopreservation solution is highly cytotoxic. This step is very important for the embryo's good survival rate.]
3. Wash the empty tube with 0.4 to 0.5 mL of SPB1.
4. Collect the embryos from the solution of Step 2 and Step 3, and transfer them into one-drop of mR1ECM on petri dish, which is prepared in Step2 of "Preparation". Keep them at 37C for 10 min.
5. Wash the surviving embryos two times with two drops of mR1ECM, remaining on petri dish. One drop for one wash.  
[You can culture the derived 2-cell embryos in the same drop of mR1ECM, without any medium change, up to blastocyst stage.]

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【JAPAN】  
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,  
KOTO-KU. TOKYO 135-0016, JAPAN  
Phone: +81-3-5632-9610  
FAX: +81-3-5632-9619  
URL: <https://www.cosmobio.co.jp/>



【Outside Japan】  
2792 Loker Ave West, Suite 101  
Carlsbad, CA 92010, USA  
email: [info@cosmobiousa.com](mailto:info@cosmobiousa.com)  
URL: [www.cosmobiousa.com](http://www.cosmobiousa.com)  
Phone/FAX: (+1) 760-431-4600