

Client Tank and Specimen Handling Instructions (IVF and ICSI)

A Receipt of package

These are 0.2mL-0.5mL IUI-processed (washed) vials. Although they are clinically suitable for an IUI procedure, the vials contain a target concentration of ~4 million motile sperm, making them better suited for IVF or ICSI procedures. See **sample utilization instructions** for IVF/ICSI prep in the attached.

General information

California Cryobank ships specimens in “dry” shippers that have been validated (at room temperature in an upright position) to maintain the temperature of the specimens below -150°C during the shipping process. Take proper safety precautions when handling the shipper and/or specimens: wear eye protection and gloves to protect from biohazard and from the extremely cold temperature created by liquid nitrogen vapor within the dry shipper.

! DO NOT TOUCH THE INNER CONTENTS WITH A BARE HAND.

Initial receipt

Upon the initial receipt of the package, **IMMEDIATELY** check the condition of the tank and perform an inventory of the specimen in the following manner.

Check the tank conditions as follows:

- 1 Open the box, but do not remove the tank from the box; save the box for the return shipment.
- 2 Verify that there is a blue sealed strap securing the tank lid to the tank, and then cut the blue strap to gain access to the tank.
- 3 Lift the cap off the tank by raising it straight up (do not twist).
- 4 Visually observe the presence of cold vapor (smoke) when the lid is removed from the tank.

Check the specimen inventory as follows:

- 1 Compare the shipping document to the actual specimen label(s).
- 2 Verify that the item number, the item description, and the quantity received are correct.

! If any of the following conditions exist:

- The blue strap seal is broken or missing upon receipt
- The tank does not have the presence of cold vapor (smoke) upon initial opening of the shipper
- The specimen item numbers on the shipping document do not match the specimen labels

Continue to transfer specimens as instructed below and notify California Cryobank immediately (866-927-9622). Do not use the specimen(s) unless approved by California Cryobank.

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B Thawing and utilization of specimen

Thawing instructions

For laboratory use:

Thaw the vials at 37°C / 98°F for 10 minutes in a water bath or 15 minutes in a dry heat block (13mm holes, minimal air around cryovial). When the specimen is completely thawed, mix the sample by slowly pipetting the entire volume up and down with a positive pressure disposal device. Do not invert the vial. Inversion can cause contents to be displaced into the vial cap, leading to partial loss of the sample.

Sample utilization

Sperm washing step

- 1 Use a positive pressure pipettor to slowly withdraw the entire volume of sample from the vial and transfer into a conical tube.
- 2 Add 3mL of HEPES-buffered sperm washing medium with added 5% (v/v) protein buffer to the conical tube containing the thawed sample.
- 3 Centrifuge at 300g for 6 minutes.
- 4 Remove the supernatant from the conical tube without disturbing the pellet, leaving approximately 0.1mL of supernatant overlaying the sperm pellet.
- 5 Re-suspend the pellet in the medium by delicately tapping the bottom of the conical tube.

Isolation step (swim up)

- 1 Carefully layer 1mL of fresh culture medium to the washed sperm mixture without mixing.
- 2 Incubate the conical tube in a tri-gas or bi-gas incubator at a 45° angle for a minimum of 20 minutes.
- 3 Carefully return the tube to a vertical position.
- 4 Use a sterile pipette to aspirate downwards from the upper meniscus to remove 1mL of the supernatant.
- 5 Transfer the aspirated supernatant to a sterile tube and return to incubator until use.

For conventional IVF procedures, a final dilution of 75,000–175,000/mL of motile sperm in a bicarbonate-buffered media should be prepared.

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Tank return

The client is responsible for returning the tank to California Cryobank. If the tank was received or left at a physician's office, the client must make arrangements with the physician's office for the tank return.

To coordinate tank return, please call client services at **866-927-9622** for assistance and tank return options. To avoid a late-return fee, the tank must be returned or picked up by our designated courier on or before the deadline indicated on the packing slip/shipment document. Failure to return the tank will result in a replacement fee of \$700.

Tank return

You have been provided with a plastic pouch and a FedEx return airbill.

- 1** Place the airbill into the plastic pouch and attach it to the top of the box. It is recommended that you retain a copy of the shipping documents for your records.
- 2** Call FedEx at the phone number provided on the enclosed shipping document to request tank pickup.
- 3** Record the tracking number for your records.