



DIRECTIONS FOR USE FOR OOCYTE THAWING MEDIUM - CHOLINE SUBSTITUTED CRYOPRESERVATION

Oocyte Thawing Medium Kit

For laboratory procedures only

Product Description	REF Number	Kit Size
Oocyte Thawing Medium Kit - Choline Substituted Cryopreservation	ART-8018	Sufficient for four (4) thawing procedures

INTENDED USE

Oocyte Thawing Medium Kit was developed for thawing of mature metaphase II oocytes frozen with ART-8017, Oocyte Freezing Medium, Choline Substituted Cryopreservation.

DESCRIPTION

The components of this kit will allow for the efficient thawing of mature metaphase II oocytes. The components and recommended procedures are the preferred method for improved oocyte survivability.

MATERIALS PROVIDED IN THE THAWING KIT

1. 4 X 2-mL vials of Oocyte Thawing Medium 0.5 M Sucrose (REF # ART-8018-A)
2. 4 x 2-mL vials of Oocyte Thawing Medium 0.2 M Sucrose (REF # ART-8018-B)

3. 4 x 4-mL vials of Thawed Oocyte Wash Medium HEPES Buffered HTF (REF # ART-8018-C)

STORAGE INSTRUCTIONS AND STABILITY

Store unopened containers refrigerated at 2°C to 8°C. Warm to room temperature (22-24°C) or incubator (37°C) temperature, as appropriate, prior to use. Do not freeze or expose to temperatures greater than 39°C. The product is stable in unopened containers until the expiration date shown on the label.

1. Remove desired volume of product using aseptic procedures. One vial contains adequate volume of medium for one thawing procedure.
2. Once removed, do not return any volume of product to the original container. Discard remaining product.
3. Do not use if the product becomes discolored, cloudy, turbid, or shows any evidence of microbial contamination.

RECOMMENDED PROCEDURES FOR CRYOPRESERVATION OF OOCYTES PRE-PROCEDURE SET-UP

1. Remove one bottle each of Oocyte Thawing Medium 0.5 M Sucrose (REF # ART-8018-A), Oocyte Thawing Medium 0.2 M Sucrose (REF # ART-8018-B), and Thawed Oocyte Wash Medium HEPES Buffered HTF (REF # ART-8018-C) from storage at 2 – 8°C and warm to room temperature (22-24°C).
2. For each thawing procedure, set up four dishes. The first dish contains 2 mL of the Oocyte Thawing Medium 0.5 M Sucrose,

the second dish contains 2 mL of the Oocyte Thawing Medium 0.2 M Sucrose, and the 3rd and 4th dish both contain 2 mL of the Thawed Oocyte Wash Medium HEPES Buffered HTF. The contents of each dish is covered with Sterile Oil for Tissue Culture (REF # ART-4008).

THAWING PROTOCOL

1. If oocytes were frozen in straws, they should be thawed rapidly (at least 275°C/min). Initially hold the straw in air for 30 to 40 seconds and then immerse it in a water bath at 30°C to 35°C until the ice has fully melted. Vials are thawed by immersion in a 32°C water bath until all ice crystals have disappeared. Thaw only one cryocontainer at a time.
2. Transfer the liquid contents of the thawed medium to a dry dish and quickly locate the oocytes. Pick the oocytes up in a minimal amount of medium and transfer them first to 2 mL of Oocyte Thawing Medium 0.5 M Sucrose (REF # ART-8018-A) at ambient temperature (22-24°C) for 10 minutes.
3. Then, using a fine-bore pipette, transfer the oocytes from the Oocyte Thawing Medium 0.5 M Sucrose to 2 mL of Oocyte Thawing Medium 0.2 M Sucrose (REF # ART-8018-B) at ambient temperature for 10 minutes, using a new transfer pipette for each procedure to minimize the carry-over of cryoprotectant from one solution to the next.
4. The oocytes are then washed through two dishes containing 2 mL of Thawed Oocyte Wash Medium – HEPES Buffered HTF (REF # ART-8018-C) at 37°C, holding in each solution for 5 minutes. The oocytes are thoroughly washed by pipetting up and

down several times over a period of about 1 minute in each solution. A new transfer pipette should be used for the first wash solution but the same pipette can be used for subsequent transfers.

5. After the second wash solution, the oocytes are rinsed through 3 x 30 uL drops of Quinn's Advantage Fertilization Medium (REF # ART-1020) containing 5 mg/mL Human Serum Albumin (REF # ART-3001) under Oil for Tissue Culture (REF # ART-4008) that have been equilibrated for a minimum of 4 hours but preferably overnight in an atmosphere of 5% CO₂; 5% O₂; 90% N₂ at 37°C, and then incubated for 3-4 hours in a fresh drop of this equilibrated medium. The oocytes are then subjected to ICSI.

Each laboratory should make its own determination of the particular details to use for each particular procedure.

Information on specific aspects of IVF, embryo culture, and cryopreservation is available in our Product Catalog (REF #80572).

One-cell MEA tested and passed with 80% or greater blastocyst. USP Endotoxin gel clot tested and passed with <0.5 EU/mL.

A Certificate of Analysis is available for this product.

PRECAUTIONS AND WARNINGS

Do not use medium that shows evidence of particulate matter, cloudiness, or is not rose colored. To avoid problems with contamination, practice aseptic technique and discard excess medium remaining in the bottle.

This product contains albumin, a derivative of human blood. All donors used in its manufac-

ture were individually tested and found to be nonreactive for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis C virus (HCV) and human immunodeficiency virus (HIV) by approved testing methods. Donors of the source material have been screened for Creutzfeldt-Jakob disease (CJD). Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of CJD is also considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.

On average, preliminary results obtained so far indicate that one may expect 63% of frozen thawed mature oocytes to survive (data on file)

REFERENCE: Boldt et al. Human Reprod 18:1250-5, 2003.

CAUTION: Investigational Device limited by U.S. Federal law to investigational use.

RELATED PRODUCTS

SAGE Assisted Reproduction Products™ has a full line of products for the Reproductive Medicine Specialist. Please call or write for specific information or to receive a copy of our current catalog. For technical questions, or to reach our Customer Service Department, call the SAGE Support Line at the number below.

Quinn's Advantage® is a registered trademark of CooperSurgical, Inc.



Call the SAGE SUPPORT LINE at:
1-800-243-2974 or 1-203-601-5200
SAGE In-Vitro Fertilization, Inc. A
CooperSurgical Company
95 Corporate Drive, Trumbull, CT 06611
www.coopersurgical.com