

Media

General Procedure

The following is a general procedure for using Cook IVF Media products. Each laboratory should establish procedures and protocols that are optimized for the individual medical facility.

For more information on the recommended use of Cook IVF Media products, please see the Cook Medical Embryo Culture - Suggested Laboratory Protocols. The manual is available upon request from Cook Medical.

Specifications and Quality Assurance

These products are supplied 'STERILE'. These products are sterilized by terminal filtration to give a sterility assurance level (SAL) of 10^{-3} .

Each lot of product is tested for:

- Endotoxin by LAL
- Biocompatibility by MEA
- Osmolality and pH (with the exception of K-SICO-50, K-SICO-200 and K-SIPV-200-5)
- Bioburden

All results are provided on lot specific Certificate of Analysis, available upon request.

Storage and Stability

When stored as directed Cook IVF Media products are stable until the expiration date shown on the vial label. These products cannot be re-sterilized after opening. Discard after use.

DO NOT USE PRODUCT IF:

- Packaging appears damaged or the seal is broken
- Solution appears turbid
- Expiry date has been exceeded

Precautions

All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested for antibodies to HIV, HbC, HCV and non-reactive for HbsAg, HCV RNA and HIV-1 RNA and syphilis. Donors of the source material have been screened for CJD. No known test methods can offer assurances that products derived from human blood will not transmit infectious agents.

Data collected to date has shown acceptable performance and safety of IVF for the treatment of sub-fertile patients. However, the long-term risk of these products and treatments is currently unknown. Therefore, any IVF procedure must occur in the context of appropriately informed patient consent.

Federal (USA) law restricts this device to sale by or on the order of a physician.

Contraindications

Products contain gentamycin (with the exception of Sydney IVF Culture Oil - K-SICO-50/200). Gentamycin should not be used on a patient that has a known allergy to gentamycin or similar antibiotics.



Gamete

Sydney IVF Follicle Flush Buffer K-SIFB-100

Intended Use

Follicle Flush Buffer is intended to be used in the removal of oocytes from ovarian follicles and to reduce the stress of the oocytes during this procedure.

General Information

Follicle Flush Buffer is supplemented with gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Follicle Flush Buffer must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Warm the Follicle Flush Buffer to 37 °C prior to use.

Sydney IVF Gamete Buffer K-SIGB-20, K-SIGB-50 & K-SIGB-100

Intended Use

Gamete Buffer is used to physically wash gametes in preparation for the fertilization step in the IVF process

General Information

Gamete Buffer is supplemented with Human serum albumin (10 mg/mL) and gentamycin (0.01 mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Gamete Buffer must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Gamete Buffer is suitable for both open and micro culture. If using micro drops ensure Culture Oil (K-SICO) is used to avoid evaporation.
- Warm the Gamete Buffer to 37 °C prior to use.

Sydney IVF Sperm Cryopreservation Buffer K-SISC-20

Intended Use

Sperm Cryopreservation Buffer protects sperm from damage resulting from ice crystal formation during freezing and long-term storage.

General Information

Sperm Cryopreservation Buffer is supplemented with Human serum albumin (10mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Sperm Cryopreservation Buffer must be stored in original unopened container, frozen at -20 °C.

Directions for Use

Freezing:

- Aseptic technique should be used.
- Ensure both the sample and Sperm Cryopreservation Buffer (K-SISC) are at room temperature.
- Mix two volumes of Sperm Cryopreservation Buffer to one volume of sample.
- Leave for 10 minutes at room temperature.
- Label straws with relevant information.
- Load the sample into a freezing straw or cryovial and seal according to manufacturer instructions.

Thawing:

- Aseptic technique should be used.
- Remove frozen samples and place at room temperature until thawing is complete.
- Open the straws in accordance with the manufacturer and remove the thawed semen.
- Dilute the semen with Gamete Buffer (KSIGB) (1:1) to reduce the toxicity of the glycerol.
- Quickly evaluate the survival of the sperm and if necessary thaw additional straws.
- Immediately prepare sperm by the density gradient method using Sperm Gradient (K-SISG) or Spermient (K-SISP) or by the swim-up procedure using Gamete Buffer (K-SIGB).

Sydney IVF Sperm Gradient K-SISG-20 & K-SISG-50

Intended Use

Sperm Gradient Kit is used to separate sperm based on density, using density gradient solutions.

General

The solutions are supplemented with Human serum albumin (10mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Sperm Gradient Kit must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Semen should be processed within one hour of collection.
- Gradients should be prepared immediately prior to use.
- Raw semen should not be centrifuged at any time.
- Allow the semen to liquefy at 37 °C for 30 minutes.
- Warm the Sperm Gradient Kit to 37 °C for a minimum of four hours prior to use.
- Prepare two gradients by adding 1.5 mL of 40 % under laid with 1.5 mL of 80 % in conical bottom tubes.
- Load 60 % or up to 2.0 mL of the ejaculate onto one gradient and 40 % or up to 1.2 mL onto the other.
- Centrifuge 20 minutes at 300 g then carefully remove the seminal plasma, the upper interface, the 40 % layer and the lower interface. Leave the remainder of the 80 % intact.
- Recover the sperm pellet using a clean pasteur pipette and re-suspend in 3 mL of Gamete Buffer (K-SIGB).
- Centrifuge for 10 minutes at 600 g.
- Repeat the washing step in a further 3 mL of Gamete Buffer (K-SIGB).
- Remove the supernatant and re-suspend the pellet in a small volume (approximately 200 µL) of Sperm Medium (K-SISM) or Fertilization Medium (K-SIFM).
- Count sperm and calculate the concentration. Adjust as required.
- Store in a 6 % CO₂ incubator at 37 °C until required.

Sydney IVF Spermient K-SISP-20 & K-SISP-100

Intended Use

Spermient is used to separate sperm based on density, using density gradient solutions.

General Information

Spermient is supplemented with Human serum albumin (10mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Spermient must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Semen should be processed within one hour of collection.
- Spermient must be diluted with Gamete Buffer (KSIGB) to an appropriate concentration for sperm preparation (e.g. 40 % and 80 % gradients).
- Gradients should be prepared immediately prior to use.
- Raw semen should not be centrifuged at any time.
- Allow the semen to liquefy at 37 °C for 30 minutes.
- Warm the Spermient to 37 °C for a minimum of 4 hours prior to use.
- Prepare two gradients by adding 1.5 mL of 40 % under laid with 1.5 mL of 80 % in conical bottom tubes.
- Load 60 % or up to 2.0 mL of the ejaculate onto one gradient and 40 % or up to 1.2 mL onto the other.
- Centrifuge 20 minutes at 300 g then carefully remove the seminal plasma, the upper interface, the 40 % layer and the lower interface. Leave the remainder of the 80 % intact.
- Recover the sperm pellet using a clean pasteur pipette and re-suspend in 3 mL of Gamete Buffer (KSIGB).
- Centrifuge for 10 minutes at 600 g.
- Repeat the washing step in a further 3 mL of Gamete Buffer (K-SIGB).
- Remove the supernatant and re-suspend the pellet in a small volume (approximately 200 µL) of Sperm Medium (K-SISM) or Fertilization Medium (K-SIFM).
- Count sperm and calculate the concentration. Adjust as required.
- Store in a 6 % CO₂ incubator at 37 °C until required.

Sydney IVF Sperm Medium K-SISM-20, SISM-50 & K-SISM-100

Intended Use

The Sperm medium is used to provide a "liquid" and nutritious environment for the sperm to maintain its motility for the "swim up" procedures and the following fertilization process.

General Information

Sperm Medium is supplemented with Human serum albumin (10 mg/mL) and gentamycin (0.01 mg/mL).

Ready to use after equilibration to 37 °C and 6 % CO₂.

Storage and Stability

Sperm Medium must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Semen should be processed within one hour of collection.
- Warm the Sperm Medium to 37 °C and equilibrate in a 6 % CO₂ incubator for a minimum of 4 hours prior to use.
- Allow the semen to liquefy at 37 °C for 30 minutes.
- Gently underlay aliquots (100 -300 µL) of fully liquefied semen under 0.5 mL aliquots of equilibrated Sperm Medium in round bottom 5 mL tubes.
- Place tubes in a test tube rack so that the tubes are at 60° to the horizontal. Place the rack in the CO₂ incubator.
- Remove the rack after 20 - 60 minutes and remove the medium above the semen (approximately 0.25 mL).
- Add 5.0 mL of equilibrated Sperm Medium to the aspirated sample and centrifuge at 600 g for 10 minutes.
- Remove the pellet and re-suspend in a small volume of equilibrated Sperm Medium or Fertilization Medium (K-SIFM).
- Count sperm and calculate the concentration. Adjust as required. Store in a 6 % CO₂ incubator at 37 °C until required.



Fertilization

Sydney IVF Culture Oil K-SICO-50 & K-SICO-200

Intended Use

Culture Oil is intended to be used as an oil overlay for culture of gametes, zygotes, or embryos in assisted reproductive technology (ART) and micro manipulation procedures. It is used to reduce osmotic stress due to evaporation and to reduce pH fluctuations.

General Information

Culture Oil is washed during the manufacturing process with Cleavage Medium (K-SICM) which is supplemented with Human serum albumin (5 mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Culture Oil must be stored in the original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Culture Oil is used for micro droplet culture from fertilization to the blastocyst. It can also be used while performing ICSI, assisted hatching and embryo biopsy.
- Pipette the intended volume of medium to be used for the micro drop on the bottom of a Petri dish.
- Gently pipette the Culture Oil into the Petri dish until the micro drops are sufficiently covered in oil.
- Equilibrate Culture Oil and bicarbonate buffered media micro drop in 6 % CO₂ at 37 °C for a minimum of 4 hours prior to use.
- Warm Culture Oil and HEPES buffered media to 37 °C before use.

Sydney IVF Fertilization Medium K-SIFM-20, K-SIFM-50 & K-SIFM-100

Intended Use

Fertilization Medium is intended to be used to provide a suitable environment for both oocytes and sperm, to promote optimal fertilization rates.

General Information

Fertilization Medium is supplemented with Human serum albumin (5mg/mL) and gentamycin (0.01mg/mL) both of pharmaceutical grade.

Ready to use after equilibration to 37 °C and 6 % CO₂.

Storage and Stability

Fertilization Medium must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Fertilization Medium is suitable for both open and micro culture. If using micro drops ensure culture oil (K-SICO) is used to avoid evaporation.
- A suitable wash volume must also be prepared.
- Fertilization medium is suitable for a standard sperm exposure (16-18 hours) or a short insemination.
- Warm the Fertilization Medium to 37 °C and equilibrate in a 6 % CO₂ incubator for a minimum of 4 hours prior to use.
- Following oocyte and sperm preparation add sperm to each well and return the dish to the incubator until fertilization check.
- Subsequent zygotes are transferred to Cleavage Medium (K-SICM).

Sydney IVF PVP K-SIPV-200-5

Intended Use

PVP is used during microinjection techniques for reducing sperm motility to enable easier capture using a pipette.

General Information

PVP is supplemented with Human serum albumin (10mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C and 6 % CO₂.

Storage and Stability

PVP must be stored in original unopened container, frozen at -20 °C.

Directions for Use

- Aseptic technique should be used.
- Warm the PVP to 37 °C and equilibrate in a 6 % CO₂ incubator for a minimum of 4 hours prior to use.
- PVP is suitable for both open and micro culture. If using micro drops ensure Culture Oil (K-SICO) is used to avoid evaporation.
- PVP consistency can be reduced by addition of Sperm Medium (K-SISM) if required.
- For procedures that will be completed in less than 10 minutes, the injection dish should be prepared with Cleavage Medium (K-SICM) warmed to 37 °C and equilibrated in a 6 % CO₂ incubator for a minimum of 4 hours prior to use.
- Alternately, if the procedure will take longer than 10 minutes, the injection dish should be prepared with Gamete Buffer (K-SIGB) warmed to 37 °C in air for a minimum of 4 hours prior to use.



Cleavage

Sydney IVF Cleavage Medium K-SICM-20, K-SICM-50 & K-SICM-100

Intended Use

Cleavage Medium is intended to be used to provide necessary nutrients for embryo development in vitro. The embryos will remain in this solution for 2 days prior to being transferred to the uterus or grown for another 3 days in Blastocyst medium.

General information

Cleavage Medium is supplemented with Human serum albumin (5mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C and 6 % CO₂.

Storage and Stability

Cleavage Medium must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Cleavage Medium is suitable for both open and micro culture. If using micro drops ensure Culture Oil (K-SICO) is used to avoid evaporation.
- A suitable wash volume must also be prepared to wash the embryo of Fertilization Medium (K-SIFM).
- Place the Cleavage Medium in an incubator with a 6 % CO₂ environment at 37 °C for a minimum of 4 hours prior to use.
- Following fertilization, transfer embryo to a culture dish containing the pre-warmed equilibrated Cleavage Medium. Wash the embryo before placing it in the final drop/well.
- The reproductive specialist may continue growth until transfer on Day 3 or transfer to Blastocyst Medium (K-SIBM) for further growth on Day 3 up to Day 6.

Sydney IVF Embryo Biopsy Medium K-SIEB-20

Intended Use

Embryo Biopsy Medium facilitates the removal of genetic material from embryonic cells, for pre-implantation genetic diagnosis.

General Information

Embryo Biopsy Medium is supplemented with Human serum albumin (5mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C and 6 % CO₂.

Storage and Stability

Embryo Biopsy Medium must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- This procedure is usually performed on day 3 embryos, before blastomere compaction occurs and is performed early in the morning.
- Warm the Embryo Biopsy Medium to 37 °C and equilibrate in a 6 % CO₂ incubator for a minimum of 4 hours prior to use.
- Ensure Culture Oil (K-SICO) is used to avoid evaporation.
- Place embryos in Embryo Biopsy Medium for a minimum of one minute and a maximum of 10 minutes.
- Biopsy the embryos in Embryo Biopsy Medium and then return the embryos back into the Blastocyst Medium (K-SIBM) with appropriate washing.

Sydney IVF Cryopreservation Kit K-SICS-5000

Intended Use

The Cryopreservation kit protects the embryo from cellular damage resulting from ice crystal formation during freezing and long term storage. It is optimized for use with 1-cell to 8-cell embryos.

General Information

This kit is designed for use with Thawing Kit (KSITS- 5000). Cryopreservation Kit is supplemented with Human serum albumin (up to 12mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Cryopreservation Kit must be stored in original unopened container, refrigerated at 2-8 °C.

Directions for Use

Preparation

- Aseptic technique should be used.
- Equilibrate Freeze Solution 1 for 10 minutes at 37 °C in air. Equilibrate Freeze Solutions 2 and 3 at room temperature for 10 minutes prior to use.

Method

- Select embryos with less than 30 % fragmentation and at the appropriate developmental stage for cryopreservation.
- Place the selected embryos directly from their culture medium into the pre-warmed Freeze Solution 1 for 10 minutes at room temperature.
- Label the freeze straws.
- Move the embryos in to Freeze Solution 2 for 10 minutes at room temperature.
- Move the embryo into Freeze Solution 3 and immediately start to load them into the prepared straws in accordance with the manufacturer.
- Place the straws in the freezing machine and initiate the freeze program suitable for cleavage stage embryos.
- Store cryopreserved embryo in liquid nitrogen.

Sydney IVF Thawing Kit K-SITS-5000

Intended Use

The Thawing kit protects the embryo from cellular damage during the thawing process following cryopreservation.

General Information

This kit is designed for use with Cryopreservation Kit (K-SICS-5000). Thawing Kit is supplemented with Human serum albumin (up to 12mg/mL) and gentamycin (0.01 mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Thawing Kit must be stored in the original unopened container, refrigerated at 2-8 °C.

Directions for Use

Preparation

- Aseptic technique should be used.
- Equilibrate the Thaw Solutions to room temperature for 10 minutes.
- Prepare a suitable volume of Cleavage Medium (KSICM) in an incubator with a 6 % CO₂ environment at 37 °C for a minimum of 4 hours prior to use, for culture of the embryo post thaw.

Method

- Thaw the straws in air for 40 seconds and then in a water bath at 30 °C for a further 30 seconds.
- Expel the straw contents into a Petri dish.
- Recover the embryo as soon as possible and place in Thaw Solution 1.
- Allow the embryo to equilibrate for 5 minutes sequentially in each of the remaining Thaw Solutions.
- When the embryo has been in Thaw Solution 4 for 5 minutes, move the dish to 37 °C for a further 5 minutes.
- Transfer the embryos to equilibrated Cleavage Medium (K-SICM) and incubate until transfer.



Blastocyst

Sydney IVF Blastocyst Medium K-SIBM-20 & K-SIBM-50

Intended Use

Blastocyst Medium is intended to be used to provide necessary nutrients for embryo development in vitro to the blastocyst stage. The embryos remain in this solution for up to 3 days prior to being transferred to the uterus.

General Information

Blastocyst Medium is supplemented with Human serum albumin (5mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C and 6 % CO₂.

Storage and Stability

Blastocyst Medium must be stored in original unopened container, refrigerated at 2-8 °C. Do not freeze.

Directions for Use

- Aseptic technique should be used.
- Blastocyst Medium is suitable for both open and micro culture. If using micro drops ensure Culture Oil (K-SICO) is used to avoid evaporation.
- A suitable wash volume must also be prepared to wash the embryo of Cleavage Medium (K-SICM).
- Warm the Blastocyst Medium to 37 °C and equilibrate in a 6 % CO₂ incubator for a minimum of 4 hours prior to use.
- After achieving desired developmental stage on day 3, transfer embryo(s) to a culture dish containing the pre-warmed equilibrated Blastocyst Medium.
- Wash the embryo(s) before placing it in the final drop/well and returning to 37 °C and 6 % CO₂ incubator.
- The reproductive specialist may continue growth until transfer on day 4 to 6.

Sydney IVF Blastocyst Cryopreservation Kit K-SIBF-5000

Intended Use

Blastocyst Cryopreservation kit protects the embryo from cellular damage resulting from ice crystal formation during freezing and long-term storage. It is optimized for use with small cells of blastocysts.

General Information

This kit is designed for use with Blastocyst Thawing (K-SIBT-5000). Blastocyst Cryopreservation kit is supplemented with Human serum albumin (up to 12mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Blastocyst Cryopreservation must be stored in original unopened container, refrigerated at 2-8 °C.

Directions for Use

Preparation

- Aseptic technique should be used.
- Equilibrate Freeze Solution 1 for 10 minutes at 37 °C in air.
- Equilibrate Freeze Solutions 2 and 3 at room temperature for 10 minutes prior to use.

Method

- Place the selected embryo directly from their culture medium into the pre-warmed Freeze Solution 1 for 10 minutes at room temperature.
- Label the freeze straws.
- Move the embryo in to Freeze Solution 2 for 10 minutes at room temperature.
- Move the embryo into Freeze Solution 3 and immediately start to load them into the prepared straws in accordance with the manufacturer.
- Place the straws in the freezing machine and initiate the freeze program suitable for blastocyst cryopreservation.
- Store cryopreserved blastocyst in liquid nitrogen.

Sydney IVF Blastocyst Thawing Kit K-SIBT-5000

Intended Use

Blastocyst Thaw protects the embryo from cellular damage during the thawing process. It is optimized for use with small blastocyst cells.

General Information

This kit is designed for use with Blastocyst Cryopreservation (K-SIBF-5000). Blastocyst Thawing is supplemented with Human serum albumin (up to 12mg/mL) and gentamycin (0.01mg/mL).

Ready to use after equilibration to 37 °C.

Storage and Stability

Blastocyst Thawing must be stored in original unopened container, refrigerated at 2-8 °C.

Directions for Use

Preparation

- Aseptic technique should be used.
- Equilibrate the four Thawing Solutions to room temperature for 10 minutes.
- Prepare a suitable volume of Blastocyst Medium (K-SIBM) in an incubator with a 6 % CO₂ environment at 37 °C for a minimum of 4 hours prior to use, for culture of the blastocyst post thaw.

Method

- Thaw the straws in air for 40 seconds and then in a water bath at 30 °C for a further 30 seconds.
- Expel the straw contents into a Petri dish.
- Recover the embryo as soon as possible and place in Thaw Solution 1.
- Allow the embryo to equilibrate for 10 minutes sequentially in each of the remaining solutions.
- When the embryo has been in Thaw Solution 4 for 10 minutes, move the dish to 37 °C for a further 10 minutes.
- Transfer the embryo to equilibrated Blastocyst Medium (K-SIBM) and incubate until transfer.



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