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.

The media is intended to be used by Embryologist/Andrologists or medical specialists who have received specialized training in embryology and laboratory techniques. The user of these products should read and understand the Instructions for Use and Precautions, and be trained in the correct procedure before performing the procedure.

SPECIFICATIONS AND QUALITY ASSURANCE
These products are supplied ‘STERILE’. These products are sterilized by aseptic filtration.

Each lot of product is tested for:
- Endotoxin by LAL
- Biocompatibility by MEA
- Osmolality and pH (with the exception of K-SICO-50-2-AA and K-SIPV-200-5-AA)
- Sterility

All sterility results are provided on lot specific Certificate of Analysis, available upon request. Reuse or re-sterilization may create risk of contamination of culture environment and/or cause patient infection.

STORAGE AND STABILITY
Cook IVF Media products must be stored in original unopened container, refrigerated at 2-8°C. Do not freeze. 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, HBV, 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.

The long term safety of children born from IVF is unknown. Data collected to date has shown acceptable performance and safety of IVF for the treatment of sub-fertile patients. 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 gentamicin (with the exception of Sydney IVF Culture Oil - K-SICO-50-2-AA). Gentamicin should not be used on a patient that has a known allergy to gentamicin or similar antibiotics.
WASTE DISPOSAL
Media waste must be treated as biological waste and should be disposed as per the local environmental regulations.

GAMETE

SYDNEY IVF FOLLICLE FLUSH BUFFER K-SIFB-100-AA

INDICATIONS FOR USE
Follicle Flush Buffer is intended for use during in vitro fertilization procedures for follicle flushing and oocyte collection.

GENERAL INFORMATION
Follicle Flush Buffer is supplemented with gentamicin (0.01 mg/mL). Ready to use after equilibration to 37°C.

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-AA, K-SIGB-50-AA & K-SIGB-100-AA

INDICATIONS FOR USE
Gamete Buffer is used to physically wash and store gametes in preparation for the fertilization step in the IVF process.

GENERAL INFORMATION
Human Sperm Survival Assay (HSSA) is performed on each lot of product. Gamete Buffer is supplemented with Human serum albumin (10 mg/mL) and gentamicin (0.01 mg/mL). Ready to use after equilibration to 37°C.

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.
• Gamete Buffer may be used as a gamete storage medium for up to 2 hours.

SYDNEY IVF SPERM GRADIENT K-SISG-20-AA & K-SISG-50-AA

INDICATIONS FOR USE
Sperm Gradient Kit is intended for use to separate sperm based on density, using density gradient solutions.

GENERAL INFORMATION
The solutions are supplemented with Human serum albumin (10 mg/mL) and gentamicin (0.01 mg/mL). Ready to use after equilibration to 37°C.
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-AA & K-SISP-100-AA

INDICATIONS FOR USE

Sydney IVF Spermient is intended for use to separate sperm based on density, using density gradient solutions.

GENERAL INFORMATION

Spermient is supplemented with Human serum albumin (10 mg/mL) and gentamicin (0.01 mg/mL). Ready to use after equilibration to 37°C.
• 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 SPERM MEDIUM K-SISM-20-AA, K-SISM-50-AA & K-SISM-100-AA**

**INDICATIONS FOR USE**
The Sperm medium intended for use during in vitro fertilization procedures to process sperm.

**GENERAL INFORMATION**
Sperm Medium is supplemented with Human serum albumin (10 mg/mL) and gentamicin (0.01 mg/mL).
Ready to use after equilibration to 37°C and 6% CO₂.

**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-50-2-AA

INDICATIONS FOR 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.

GENERAL INFORMATION
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. Protect from light.

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.


INDICATIONS FOR USE
Fertilization Medium is intended for use during in vitro procedures for insemination and incubation of oocytes.

GENERAL INFORMATION
Fertilization Medium is supplemented with Human serum albumin (5 mg/mL) and gentamicin (0.01 mg/mL), both of pharmaceutical grade. Ready to use after equilibration to 37°C and 6% CO₂.

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-AA

INDICATIONS FOR USE
Sydney IVF PVP is intended for use as an aid in the immobilization and isolation of individual sperm cells prior to intracytoplasmic sperm injection (ICSI) procedures.

GENERAL INFORMATION
PVP is supplemented with Human serum albumin (10 mg/mL) and gentamicin (0.01 mg/mL). Ready to use after equilibration to 37°C and 6% CO₂.

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.
• Injection dish should be prepared with a concentric pattern of Cleavage Medium (K-SICM) droplets.
• Place a drop of PVP in the centre of the dish
• Overlay the droplets with culture oil (K-SICO) and equilibrate in a 6% CO₂ environment at 37°C prior to use. Alternatively, if a 6% CO₂ environment is not to be used, the injection dish can be prepared with Gamete Buffer (K-SIGB) and equilibrated at 37°C prior to use.
• Introduce a volume of 1-2µl of prepared sperm suspension into the centre of the PVP droplet. Wait a few minutes to allow for migration of sperm to the outer perimeter of the droplet.
• Introduce an oocyte into each of the Cleavage Medium droplets.
• Proceed with ICSI.

CLEAVAGE


INDICATIONS FOR USE
Cleavage Medium is intended for use during in vitro fertilization procedures for culture and transfer of cleavage stage embryos.

GENERAL INFORMATION
Cleavage Medium is supplemented with Human serum albumin (5 mg/mL) and gentamicin (0.01 mg/mL). Ready to use after equilibration to 37°C and 6% CO₂.
**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% CO\(_2\) 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.

**BLASTOCYST**

**SYDNEY IVF BLASTOCYST MEDIUM K-SIBM-20-AA & K-SIBM-50-AA**

**INDICATIONS FOR USE**

Blastocyst Medium is intended for use during in vitro fertilization procedures for extended culture and transfer of embryos.

**GENERAL INFORMATION**

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

Ready to use after equilibration to 37°C and 6% CO\(_2\).

**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% CO\(_2\) 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% CO\(_2\) incubator.
- The reproductive specialist may continue growth until transfer on Day 4 to 6.

**SYDNEY IVF BLASTOCYST VITRIFICATION KIT K-SIBV-5000-AA**

**INDICATIONS FOR USE**

Blastocyst Vitrification Kit is intended to be used for the vitrification of Human blastocysts for A.R.T. procedures. This kit is designed for use with Blastocyst Warming Kit (K-SIBW-5000-AA).

**GENERAL INFORMATION**

Blastocyst Vitrification Kit consists of three HEPES buffered media designed for sequential use. Vitrification solutions are supplemented with Human serum albumin (up to 20.0 mg/mL) and gentamicin (0.01 mg/mL).
WARNING: Blastocyst Vitrification Kit is not ready for use. It requires the combination of vitrification solutions by the end user prior to use. Vitrification Solution 4 is Dimethyl sulphoxide (DMSO). A material Safety Data Sheet (MSDS) is available upon request.

This product must be used with a legally marketed storage device that is indicated for use in blastocyst vitrification procedures. The storage device must be a closed storage system to prevent the potential risk of viral contamination that exists with open storage systems. Any storage device must be appropriate for blastocyst vitrification and must achieve a cooling rate of > minus 15 000 degC/min.

PRODUCT DESCRIPTION

The three media are all based upon the formulation of Cryobase buffer, a 10 mM HEPES buffered media containing 20.0 mg/mL HSA, 0.01 mg/mL gentamicin. Solution 1 contains Cryobase buffer only. Solution 2 is used for preparation for vitrification and contains Cryobase buffer with 8% ethylene glycol and 8% DMSO. Solution 3 is used during cyrostorage and contains Cryobase buffer with 0.68 M trehalose, 16% ethylene glycol and 16% DMSO. Solution 4 is a glass vial of DMSO.

DIRECTIONS FOR USE

Preparation

- Aseptic technique should be used.
- DMSO is a frozen solid at 2-8°C. Before use equilibrate Vitrification Solution 4 (DMSO) to room temperature prior to use.
- Add 400 µL of Vitrification Solution 4 (DMSO) to 4.6 mL of Vitrification Solution 2 and mix well.
- Add 1 mL of Vitrification Solution 4 (DMSO) to 5.25 mL of Vitrification Solution 3 and mix well.
- Prepare the vitrification solutions in a 4 well dish by adding 800 µL of Vitrification Solution 1 into well 1 and 2, 800 µL of the prepared Vitrification Solution 2 into well 3, and 800 µL of the prepared Vitrification Solution 3 into well 4.
- Equilibrate the three Vitrification Solutions to 37°C prior to use.

Method

- Place the embryo(s) to be cryopreserved into well 1, maximum of three embryos. Leave for a maximum of 7 minutes.
- Move the specific embryo to be vitrified into well 2. Have the allocated vitrification device ready. Leave for a maximum of 1 minute.
- Move the embryo to well 3 for two minutes.
- Move the embryo to well 4. Complete the next step within 20-30 seconds.
- Using a positive displacement pipette, aspirate the embryo in 1.5 µL of solution. Avoid bubbles.
- Expel the droplet containing the embryo onto vitrification device and vitrify immediately according to laboratory approved methods.

SYDNEY IVF BLASTOCYST WARMING KIT K-SIBW-5000-AA

INDICATIONS FOR USE

Blastocyst Warming Kit is intended for the warming of Human blastocysts that have undergone vitrification using Blastocyst Vitrification Kit (K-SIBV-5000-AA) for A.R.T. procedures.

GENERAL INFORMATION

Blastocyst Warming Kit consists of three HEPES buffered media designed for sequential use. Warming solutions are supplemented with Human serum albumin (up to 20.0 mg/mL) and gentamicin (0.01 mg/mL).
PRODUCT DESCRIPTION

The three media are all based upon the formulation of Cryobase buffer, a 10 mM HEPES buffered media containing 20.0 mg/mL HSA, 0.01 mg/mL gentamicin. Solution 1 is the first medium used in the thawing process and contains Cryobase buffer with 0.33 M Trehalose. Solution 2 contains Cryobase buffer with 0.2 M Trehalose. Solution 3 is used for recovery and contains Cryobase buffer only.

DIRECTIONS FOR USE

Preparation

- Aseptic technique should be used.
- Equilibrate the three Warming Solutions to 37°C before use.
- Prepare a suitable volume of Blastocyst Medium (K-SIBM) in an incubator with a 6% CO₂ environment at 37°C prior to use, for culture of the blastocyst post warming.

Method

- Identify the vitrification device(s) to be warmed and warm in accordance with the manufacturer.
- Prepare the Warming Solutions in a 4 well dish by adding 800 µL of Warming Solution 1 into well 1 and 2, 800 µL of Warming Solution 2 into well 3, and 800 µL of Warming Solution 3 into well 4.
- Immediately extract the embryo from the vitrification device and place into well 1 and STIR IMMEDIATELY until the bead dissolves.
- Using a pipette, transfer the embryo to well 2 for 5 minutes.
- Move to well 3 for 5 minutes. Wash the embryo well.
- Move to well 4 for 5 minutes. Wash the embryo well.
- Place the embryo in the dish containing equilibrated Blastocyst Medium (K-SIBM) ready for hatching.
- If required, hatch the embryo (laser method preferred) and move to a new micro droplet of equilibrated Blastocyst Medium (K-SIBM).