FREEZING KIT

W / Penicillin-streptomycin
W / Bovine serum albumin
W / HEPES buffer

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A. USAGE:

- For cryopreservation of sperm for further use in Assisted Reproductive Technologies.

B. CONTENTS:

1. Freezing solution **(FS)**.
2. Product insert.

C. STORAGE:

- The kit Keeps dry at **2 – 8 °C**.

D. STERILITY:

- Sterile Filtered.

E. COMPOSITION:

Inorganic salts	**Buffers**	Penicillin-streptomycin	Sucrose
Sodium Chloride	Sodium Bicarbonate	**Energy Sources**	Glycerol
Sodium Phosphate	HEPES	Dextrose	**Water**
Potassium Chloride	**Amino Acids**	**Protein**	WFI Quality
Magnesium Sulfate	Glycine	Bovine serum albumin	
Calcium Chloride	**Antibiotics**	**Cryoprotectant**	

F. PRECAUTIONS:

- Respect storage conditions of the product.
- Do not use the product after its expiry date.
- Manipulate the product in aseptic conditions (e.g.: under laminar air flow).
- Wear clothes adapted to the manipulation of the product to avoid contamination (e.g.: gloves, mask, hygiene cap, overall, etc.).

G. PROCEDURES:

- BioMEDIA provide easy cryopreservation protocol for sperms originated from ejaculate or TESE extracted in simplified way to obtain high survival rate **60 – 80 %**.

Sperm cryopreservation protocol:

1. Cryopreservation is performed on native semen samples or processed samples.
2. Ensure Freezing solution (FS) is well mixed at room temperature before use.
3. For native semen sample allow the semen to liquefy at **37 °C** for **30 minutes**.
4. Mix equal volume of semen with Freezing solution (FS).
5. Add the Freezing solution (FS) in drops gently to avoid osmolarity shock within **2 minutes** with continues mixing and leave the mixture for **10 minutes** at room temperature for equilibration.

6. Suck the sample/Freezing solution (FS) mixture into the freezing straw **(e.g. SPERM CRYO SYSTEM)**, leaving approximately **1.5 cm** of air at the end of the straw and seal the straw.
7. Place the straw horizontally in a liquid nitrogen vapor for freezing occur.
8. leave for **30 minutes**, transfer straws quickly into liquid nitrogen and store at **-196 °C**.

Sperm thawing protocol:

1. Remove straw from the liquid nitrogen and place the straw in tap water for **1 minutes (room temperature or 37 °C)**.
2. Cut the end of straw and place the open side inside a container and cut the head of straw for obtaining sample/Freezing solution (FS) mixture.
3. Dilute the sample/Freezing solution (FS) mixture in a suitable HEPES buffered media **(e.g. S-VIVO MEDIA)** at least 3 ml per **0.25 ml** or **0.5 ml** of sample/Freezing solution (FS) mixture for removing cryoprotectant.
4. Centrifuge at **300 g** for **10 minutes**.
5. Resuspend pellet in a suitable volume of HEPES buffered media **(e.g. S-VIVO MEDIA)** according to pellet size.
6. Finally, asses recovered sample.

H. TECHNICAL ASPECTS:

1. Increasing of exposure time to Freezing solution (FS) may be harmful for sperm.
2. During thawing, removed the straw quickly from liquid nitrogen and put into tap water **(room temperature or 37 °C)** to avoid heat shock.
3. Quick dilution after thawing is very important to avoid toxicity and increase survival rate.

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