WARMING KIT®

W / Penicillin-streptomycin
W / Human serum albumin

A. USAGE:

- For thawing vitrified of oocytes (MII), pronuclear (PN), zygotes through Day 3 cleavage stage embryos and blastocyst stage embryos.

B. CONTENTS:

1. 1- Thawing solution **(TS)**.
2. 2- Dilution solution **(DS)**.
3. 3- Washing solution **(WS)**.
4. 4- Product insert.

C. STORAGE:

- The kit Keeps dry at **2 – 8 °C**.

D. STERILITY:

- Sterile Filtered.

E. COMPOSITION:

Inorganic salts
Sodium Chloride
Sodium Phosphate
Potassium Chloride
Magnesium Sulfate
Sodium Acetate
Calcium Chloride
Choline Chloride
Ferric Nitrate

Buffers
Sodium Bicarbonate
HEPES

Amino Acids
Arginine
Glycine
Histidine
Lysine
Proline
Tyrosine
Alanine
Aspartic Acid
Glutamic Acid
Isoleucine
Leucine
Methionine
Phenylalanine
Serine
Threonine
Tryptophan
Valine
Hydroxyproline
Cystine
Cysteine

Antioxidant
Glutathione

Others
Adenine Sulfate
Deoxyribose
Ribose
Guanine
Uracil
Xanthine
Thymine
Hypoxanthine
Adenosine

Vitamins & Minerals
Calciferol
Ascorbic Acid
Aminobenzoic Acid
Nicotinic Acid
Nicotinic Acid Amide
Pantothenic Acid
Riboflavin
Thiamine
Biotin
Pyridoxine
Sodium Bisulfite
Alpha-Tocopherol
Folic Acid

Antibiotics
Penicillin-streptomycin

Energy Sources
Dextrose
Inositol

Protein
Human serum albumin

Cryoprotectant
Sucrose

Water
WFI Quality

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 warming protocol for oocytes and embryos in different stages in simplified way to obtain high survival rate **90 – 99 %**.

Oocyte / Embryo warming protocol (Fig. 3):

1. Warm the **thawing solution (TS)** vial at **37 °C** for **30 min.** at least.
2. Warm the **dilution solution (DS)** vial and the **washing solution (WS)** vial at room temperature for **30 min.** before use.
3. Gently remove the cap of vitrification device under liquid nitrogen, quickly release it from the liquid nitrogen into **400 ul** of **thawing solution (TS)** drop within **1 sec.**, immerse it fully, then swirling the vitrification device, and thawing time should not exceed **1 min.**
4. Transfer **oocytes / embryos** to **100 ul** of **dilution solution (DS)** for **3 – 5 min.**
5. Transfer **oocytes / embryos** to **50 ul** of **washing solution (WS1)** for **3 min.**
6. Transfer **oocytes / embryos** to **50 ul** of **washing solution (WS2)** for **3 min.**
7. Transfer warmed **oocytes / embryos** to equilibrated culture media supplemented with **20 %** Human serum albumin for recovery at least **2 – 3 hour** before manipulation.

H. TECHNICAL ASPECTS:

1. Avoid bubbles while dispensing the contents.
2. The **oocytes / embryos** will shrink and float to the top of the drop at **thawing solution (TS)**.
3. The **oocytes / embryos** will remain shrunken during exposure to **dilution solution (DS)**.
4. Do not begin warming procedure until you have a pre-equilibrated dish of appropriate culture media supplemented with **20 %** Human serum albumin.
5. Swirling the vitrification device is critical to ensure the most rapid thawing temperature rate **(> 24000 °C/min.)**.
6. Vitrification device must remain submerged in liquid nitrogen until ready to warm and when transferring from liquid nitrogen filled holding reservoir, or between liquid nitrogen storage tanks.

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