VITRIFICATION KIT®

W / Penicillin-streptomycin
W / Human serum albumin

A. USAGE:

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

B. CONTENTS:

1. Equilibrium solution **(ES)**.
2. Vitrification solution **(VS)**.
3. 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
Ethylene Glycol
Dimethylsulfoxide

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

Oocyte vitrification protocol [Fig. 1]:

1. Procedures under go at room temperature to avoid osmolarity Shock.
2. Put the oocytes into **50 ul** of **HEPES buffered media** drop with protein (e.g.: HTF HEPES buffered media).
3. Merge **25 ul** of **Equilibrium solution (ES1)** drop with the previous drop for **2 minutes**.
4. Merge **25 ul** of **Equilibrium solution (ES2)** drop with the previous drops for **2 minutes**.
5. Merge **25 ul** of **Equilibrium solution (ES3)** drop with the previous drops for **2 minutes**.
6. Transfer the oocytes to **50 ul** of **Equilibrium solution (ES4)** drop for **2 – 6 minutes** until equilibrium occurs.
7. Transfer the oocytes to **50 ul** of **vitrification solution (VS)** drop for **30 – 110 second**.
8. Load the oocytes with minimal volume of **vitrification solution (VS)**.
9. Plunge the vitrification device into liquid nitrogen and seal it under liquid nitrogen.
10. Store at **-196 °C** in nitrogen tank.

Embryo vitrification protocol [Fig. 2]:

1. Procedures under go at room temperature to avoid osmolarity Shock.
2. Put the embryos into **50 ul** of **equilibrium solution (ES)** drop maximum three embryos for **5 – 15 minutes** until equilibrium occurs.
3. Transfer the embryos to **50 ul** of **vitrification solution (VS)** drop for **30 – 110 second**.
4. Load the embryos with minimal volume of **vitrification solution (VS)**.
5. Plunge the vitrification device into liquid nitrogen and seal it under liquid nitrogen.
6. Store at **- 196 °C** in nitrogen tank.

H. TECHNICAL ASPECTS:

1. Load, plunge, and seal loading device within **90 seconds**, not to exceed **110 seconds** after initial exposure to **vitrification solution (VS)**.
2. Minimize exposure of **oocytes / embryos** to light during equilibration in **equilibrium solution (ES)** and **vitrification solution (VS)**.
3. Maintain microscopic visualization of **oocytes / embryos** by adjusting focus as needed during rapid exposure to **vitrification solution (VS)**.
4. The timing for exposure to **vitrification solution (VS)** is critical because exposure of **oocytes / embryos** to **vitrification solution (VS)** should be limited to prevent cytotoxicity.

***