Erythrocyte Lysis Buffer

W/Phenol Red

A. USAGE:

- For discard red blood cells from samples that contain sperm cells without effect on sperm viability such as row semen, FNA, and TESE.

B. STORAGE:

- Keep dry at 2 – 8 °C.

C. STERILITY:

- Sterile Filtered.

D. COMPOSITION:

- Erythrocyte Lysis Buffer isosmotic and aim to keep all cells in solution in osmotic balance to avoid sperm swelling.
- The active ingredient in Erythrocyte Lysis Buffer is ammonium chloride.
- The osmotic rupture of red blood cells alone occurs because of the presence of an abundant membrane Cl^- / HCO_3^- anion exchanger called Band 3 on the surface of mature red blood cells.
- Upon exposure to the Erythrocyte Lysis buffer, the chloride ions will be driven to enter the red blood cells via the exchanger because of the ion gradient.
- This will cause the HCO_3^- ions to be driven out by the exchanger.
- This causes water to rush into the red blood cells and weaken its integrity and eventually lyse it.

E. 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.).

F. PROCEDURES:

1. Incubate the Erythrocyte Lysis Buffer at 37 °C for 30 min. at least before using.
2. After processing sample underwent for mechanical fraction or squeezing, centrifugation at 300 g for 5 minutes to concentrate the sperm sample.
3. Discard supernatant before applying the incubated Erythrocyte Lysis Buffer.
4. Re-suspension by 1.5 ml incubated Erythrocyte Lysis Buffer with good mix by pasture pipette.
5. Just apply incubated Erythrocyte Lysis Buffer on target sperm sample for 5 minute.
6. Equilibrate the sperm sample by adding equal volume or more of any washing HEPES buffered media such as Ham's F – 10, and discard large debris to avoid viscosity.
7. Re-concentrate the solution by centrifugation at 300 g for 8 min. to suitable volume according to the yield.
8. Finally, the sample is ready for examination and use.

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