Sperm DNA®

Kit to asses sperm DNA fragmentation

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A. USAGE:

- To assessment sperm DNA fragmentation index (SDFI) using bright field microscopy.

B. CONTENTS:

1. Denaturation solution.
2. Lysis solution.
3. Stain (A) solution.
4. Stain (B) solution.
5. Agarose cell support.
6. Super coated slides.
7. Eppendorf tubes.
8. Float.
9. Product insert.

C. PRECAUTIONS:

1. All patient samples should be considered potentially infectious and the user must wear protective gloves, eye protection, face mask and laboratory coats when performing the test and take off contaminated parts immediately.
2. After contact with skin wash immediately with water and soap.
3. The test should be discarded properly after use **(biohazard)**.
4. Do not use after expiration date, which appears on the package label.

D. WARNINGS:

1. Care should be taken to avoid contact with skin or eyes, and to prevent inhalation.
2. Work under air removal environment.
3. Do not release the products used into the environment.

E. STORAGE:

- The kit keeps dry at **2 – 8 °C**.

F. PEPARATION OF AGAROSE:

- Place the agarose vial tube into the float and melt using a water bath or a beaker with water on a hot plate at **95 – 100 °C for 5 minutes** or until it is completely melted then quickly divide the agarose into Eppendorf tubes with **100 µl** each.

G. PREPARATION OF SLIDE: (Set the kit at room temperature during the whole process)

1. To avoid sperm crowding in high concentration sample dilute the sperm sample in an appropriate sperm wash medium to reach **20 million/ml**.
2. Take **50 µl** from sperm sample, mix with **100 µl** melted agarose at **37 °C** in Eppendorf tubes.
3. Add the **10 µl** prepared sample to the slide with coverslip then transfer to cold surface (ex. Fridge) at **4°C for 5 minutes** to solidify the agarose.
4. Take the slide out of the fridge and remove the coverslip by sliding it off gently.

H. PROCESSING THE SLIDE: (All the processing must be performed at room temperature)

1. Apply **Denaturation solution** on the slide making sure it is fully covered by the reagent during the whole process, incubate for **7 minutes**, and then remove the reagent.
2. Apply **Lysis solution** on the slide making sure it is fully immersed, incubate for **20 minutes**, and then remove the reagent.
3. **Wash** the slide with distilled water for **5 minutes**.
4. **Dehydrate** with 70 % ethanol for **2 minutes**.
5. **Drain** with 100 % ethanol for **2 minutes**.

NOTE:

Processed slides may be kept in slide boxes at room temperature in a dry and dark place for several months.

I. STAINING PROCESS:

1. Apply **Stain (A)** for **10 minutes,** aspirate the excess of stain (A) by pipette from one end of slide and let thin layer **(1:3 of amount)** of stain (A) before adding stain (B).
2. Apply **Stain (B)** for **10 minutes**, slip the slide at one side and drying gradually to removing excess of stain (B).
3. Let the slide to dry for **5 minutes**.

J. SDFI CALCULATIONS:

- Count 200 sperm or more then apply to the following calculation formula:

SDFI (%) = Fragmented sperms / Total number of sperms x 100

- **Non fragmented sperm:**
  - **Big halo:** halo width is similar or higher than the diameter of the core.
  - **Medium halo:** halo width is between those with large and with very small halo.
- **Fragmented sperm:**
  - **Small halo:** halo width is similar or smaller than 1/3 of the diameter of the core.
  - **No halo:** those that show no halo.
  - **Degraded:** those that show no halo and present a core irregularly or weakly stained.