GoldCyto Sperm DNA kit (20 Assays)

KIT TO ANALYZE SPERM DNA FRAGMENTATION IN ANIMALS

APPLICATION

The GoldCyto Sperm ®Kit from Goldcyto Biotech corp. is a simple test that allows assessment of sperm DNA fragmentation in animals.

PRINCIPLE OF THE METHOD

The method is based on the Sperm Chromatin Dispersion (SCD) test (Fernández et al., J. Androl 24:59–66, 2003; Fertil Steril 84:833-842, 2005). Intact unfixed spermatozoa (fresh, frozen/unthawed, diluted or neat samples) are immersed in an inert agarose microgel on a pretreated slide. An initial lysing treatment removes most of the nuclear proteins, and in the presence of massive DNA breakage produces nucleoids with large halos of spreading DNA loops, emerging from a central core. However, the nucleoids from spermatozoa with intact DNA either do not show a dispersion halo or the halo is minimal.

DESCRIPTION OF KIT CONTENTS

-20 Pretreated slides

-20 Eppendorf tubes with low-melting-point agarose (Agarose Cell Support)

-1 Bottle of Phosphate Buffer Saline.50 ml

-1 Bottle of Lysis solution.120 ml

-1 Bottle of stain solution-TA.25 ml

-1 Bottle of stain solution -TB.50 ml

MATERIAL AND EQUIPMENT REQUIRED

Bright field or fluorescence microscope. -4ºC fridge. 90-100ºC and 37ºC incubation bath(s). Plastic gloves. Glass slide covers (18x18 mm or 22x22 mm). Micropipettes. Trays for horizontal incubations. Distilled water. Ethanol 70%, 90%, 100%. Microwave oven and fume hood.

Recommended solutions for brightfield microscopy:
- Wright solution (Merck 1.01383.0500)
- Phosphate buffer solution pH 6.88 (Merck 1.07294.1000)
- Mounting medium: Eukitt® (Panreac 253681)

INSTRUCTIONS FOR USE

1. Set the lysis solution at room temperature (22ºC).

   Warning: Shake well before using whatever there is precipitation.

2. Dilute the semen sample in culture medium or sperm extender or PBS to a concentration of 5 – 10 million per milliliter. Be careful solvent not to be extremely dense. Both fresh samples and samples directly frozen in liquid nitrogen may be used.

3. Put the agarose eppendorf provided through a float; the float should be at the level of the top of the tube. Leave floating in water 5 minutes at 90º -100ºC, until the agarose dissolves. Alternatively, the agarose can be melted in a microwave oven.

4. Transfer the agarose eppendorf, with the float, to a temperature controlled water bath maintained at 37º C and leave for 5 minutes until the temperature is even.

5. Add 30 microliters of the semen sample to the agaro se eppendorf and mix.

   Place the cell suspension from the agarose eppendorf onto the treated side of the slide and cover with a glass coverslip, being careful to avoid air bubbles. A drop of 14 or 20 µl for an 18x18 mm or 22x22 mm coverslip respectively, is recommended. If liquid does not spread till the edge of the coverslip, press gently with the tip of the micropipette.

6. Take care to keep the slide in a horizontal position throughout the entire process.

7. Place the slide on a cold surface (for example, a metal or glass plate pre-cooled at 4ºC). Put the cold plate with the slide/s into the fridge at 4ºC and leave the sample to gel for 5 minutes

8. Remove the slide cover by sliding it gently, and immediately immerse the slide into a tray containing 10ml of lysis solution in a horizontal position, leaving it to incubate for 5 minutes at room temperature (22ºC).

9. Pick the slide up and set up horizontally into a tray containing abundant distilled water in order to wash the lysis solution. Leave to incubate for 5 minutes.

10. Place the slide horizontally into a tray with 70% ethanol (2 minutes), followed by 90% ethanol (2 minutes) and finally, 100% ethanol (2 minutes).

11. Leave to dry at room temperature and stain.

12. After drying, the processed slides may be kept in archive boxes at room temperature, in the dark, for months.

Note: As an internal control, it is recommended to process a microgel with a sample of well-known level of DNA fragmentation. Because two samples can be analyzed on the same slide, a control and sample or two different samples can be co-processed using a single slide.

Staining and visualization

13. Place the slide horizontally on the float inside the Petri dish
14. Apply Solution -TA （0.5-0.8ml ） on the slide making sure it is fully immersed .incubate for 1 minutes ,Don't drain !
15. Apply Solution –TB value =(2 or 3) x TA on the slide making sure it is full immersed , Blow with your mouth or ear ball to make the liquid surface ripple, mix TA, TB, thoroughly , incubate for 3-10 minutes ,
16. Washing (flushing) : Do not pour out the staining solution, it should be flushed away by tap water (To prevent sediment from being deposited on the specimen) while flushing briefly and smoothly in tape water , allow to dry at room temperature.
17. Check colouring level under the microscope. Strong staining is preferred, to clearly discriminate the peripheral border of the halo. If staining results very weak, especially on the region of chromatin dispersion halos, the slide can be re-stained with Wright's solution. If colouration is too strong, the slide can be discoloured by washing gently in tap water, or 10% ethanol if preferred. After air dried, it can be dyed again but reducing colouring exposure time.

Once the desired level of coloration is achieved, and the slide is perfectly dried, mount it with DPX or equivalent synthetic medium for making permanent slides.

SPERM CLASSIFICATION

Score a minimum of 300 sperm per sample following the criteria:

SPERMATOZOA WITH DNA FRAGMENTATION

- Spermatozoa with big halo: those whose halo width is similar or higher than the minor diameter of the core (Figure 1).

- Spermatozoa with medium-sized halo: their halo size is between those with large and with very small halo (Figure 2).

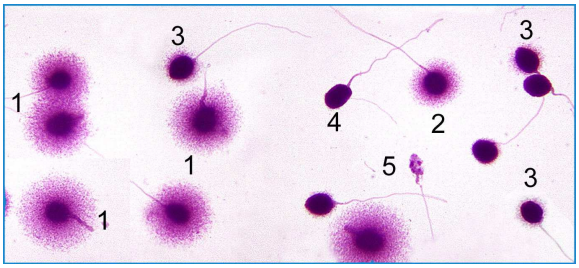
SPERMATOZOA WITHOUT FRAGMENTED DNA

- Spermatozoa with small halo: the halo width is similar or smaller than 1/3 of the minor diameter of the core (Figure 3).

- Spermatozoa without halo (Figure 4).

- Spermatozoa without halo and degraded: those that show no halo and present a core irregularly or weakly stained (Figure 5).

"Others": cell nuclei which do not correspond to spermatozoa. One of the morphological characteristics which distinguish them is the absence of tail.



INTERPRETING THE RESULTS

Calculate the percentage of sperm with fragmented DNA . The results should be evaluated taking into account all clinical and laboratory findings related to the sperm sample

Thresholds for frequency of Sperm DNA Fragmentation (SDF) have been suggested by Evenson et al. (J. Androl 23:25-43,1999)

SDF	Evaluation
<15%	Low
Between 15 and 30%	Medium
>30%	High

SAFETY AND THE ENVIRONMENT

Attention! Slide processing is recommended performing under fume hood or flow hood

Avoid inhalation and contact with the solutions supplied. The lysing solution contains Dithiothreitol, and Triton X-100. Consult specifications supplied by manufacturers.

Do not release the products used into the environment. Follow center guidelines for the storage and disposal of toxic substances. Biological samples must be handled as potentially infectious.

STORAGE AND STABILITY

Lysing solution is recommended to be stored at 4ºC and protected from light. The kit products may be at room temperature for several days without problem.

Expiration: the reagents supplied are stable for a minimum period of 12 months. It is recommended that solutions be kept well sealed and air free as much as possible.