

PREPARING THE SAMPLE

1. Select the time points (T_0 , T_1 , T_2 , T_3) you wish to analyze to construct the dynamic DNA fragmentation curve. For more details, see the instruction sheet.
2. Dilute the semen sample to a concentration of 16-20 million sperm cells per ml
3. Aliquot 25 μ l of the sample into each of the four colored eppendorf tubes
4. Freeze the T_0 aliquot at -20°C
5. Incubate the three remaining aliquots at 37°C . Remove from 37°C incubation and sequentially place each aliquot at -20°C at the chosen time points: T_1 , T_2 , and T_3

PROCESSING THE SAMPLE

6. Thaw the 4 aliquots (T_0 , T_1 , T_2 , and T_3) at room temperature and then incubate at 37°C
7. Warm one agarose eppendorf tubes for each slide to be processed until the agarose is fully liquefied. A microwave oven or hot water bath may be used
8. Cool the agarose to 37°C and mix 50 μ l of agarose into each semen aliquot
9. Place 8 μ l of the agarose-semen mixture from the T_0 aliquot onto the T_0 well on the pretreated slide
10. Place 4 μ l of the agarose-semen mixture from the T_1 , T_2 , and T_3 aliquots onto their corresponding wells on the pretreated slide

6. Place a coverslip over the slide and place the slide on a cold surface or in the refrigerator at 4°C during 5 minutes to solidify the agarose
7. Once the agarose is solid, remove the coverslip by gently sliding it off the slide using your thumb
8. Place a few drops of AD solution on each well so that they are fully immersed and incubate for 7 minutes
9. Tip the slide to remove the AD solution and place a few drops of LS solution on each well so that they are fully immersed and incubate for 20 minutes
10. Tip the slide to remove the LS solution and immerse the slide as before with distilled water and incubate for 5 minutes
11. Tip the distilled water off the slide and dehydrate the sample incubating the slide as before in 70% ethanol for 2 minutes, and 100% ethanol for another 2 minutes
12. Dry the slide at room temperature

STAINING AND VISUALIZATION UNDER THE MICROSCOPE

18. Once dry, immerse the wells in TA solution during 6 minutes
19. Remove the TA solution and immerse the slide in TB solution during 7 minutes
20. Wash briefly in water and leave to dry
21. Put a drop of water over the slide and place a coverslip on top
22. Visualize under the microscope