LensHOOKE®

R10
Sperm DNA Fragmentation Test Kit (SCD Assay)

User Manual | For Professional Use

Rev.30-0R10002
20220217

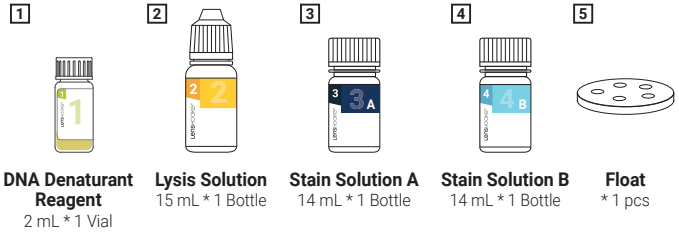
Introduction

Thank you for choosing LensHooke® Sperm DNA Fragmentation Test Kit (SCD Assay). Please read this user manual carefully before using. This product is for in vitro diagnostics only. LensHooke® Sperm DNA Fragmentation Test Kit (SCD Assay) is designed, manufactured and authorized by Bonraybio Co., LTD. to the agencies.
If you have any question, please contact with our Service Hotline: +886-4-24912385#241 (Mon.-Fri. 8AM-5PM PST).

Intended Use

The LensHooke® Sperm DNA Fragmentation Test Kit (SCD Assay) is a simple and easy-to-use assay for evaluating sperm DNA fragmentation in human semen specimens.For professional use.

LensHooke® Sperm DNA Fragmentation Test Kit Components



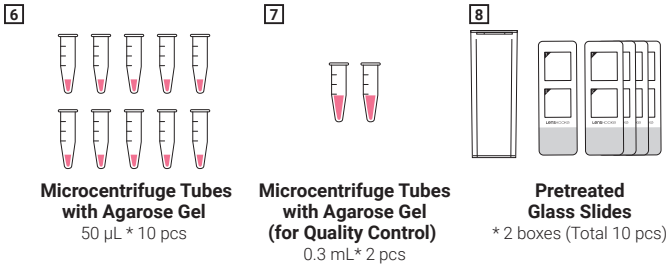
DNA Denaturant Reagent
2 mL * 1 Vial

Lysis Solution
15 mL * 1 Bottle

Stain Solution A
14 mL * 1 Bottle

Stain Solution B
14 mL * 1 Bottle

Float
* 1 pcs



Microcentrifuge Tubes with Agarose Gel
50 µL * 10 pcs

Microcentrifuge Tubes with Agarose Gel (for Quality Control)
0.3 mL* 2 pcs

Pretreated Glass Slides
* 2 boxes (Total 10 pcs)

Material and Equipment preparation (required but no provided in Kit)

1. Bright field microscope
2. Fridge at 2~8°C
3. Disposable droppers
4. Plastic gloves
5. Glass coverslip (22 × 22 mm)
6. Slide staining tray
7. Distilled water in wash bottle
8. 95% Methanol (CAS Number: 67-56-1)
9. 0.01M PBS or sperm extender
10. 95~100°C hot water
11. 200µL/1000µL Pipette
12. Dust blower

Principle of the method

- This kit is based on the sperm chromatin dispersion (SCD Assay).
- Unfixed semen sample (fresh, frozen/defrosted, diluted or neat samples) are embedded in a melted agarose microgel and attached to a pretreated glass slide.
- After DNA denaturation, lysis of nuclear proteins and staining procedures, sperm with fragmented DNA do not form the characteristic DNA halo seen in sperm with intact DNA under the microscope.

Safety and Environment

1. It is necessary to wear a protective gloves, eye protection, and laboratory coat during the entire process of the assay.
2. Perform the assay in an air ventilated environment or fume hood to avoid inhale odor derived from the lysis solution.
3. Do not directly drain the reactive used into the environment. Follow laboratory safety regulations for the storage and disposal of waste effluent.
4. All test samples must be handled as potentially infectious.

Storage and Stability

Recommended storage conditions: 2~8 °C away from light.
Shelf life after opening: Keep all components well-sealed after use, and store at 2-8 °C.The kit is stable for a minimum of 3 months after opening.

Specification

Contents	Composition
1 DNA Denaturant Reagent	Hydrochloric acid, 2 mL
2 Lysis Solution	Reducing agent, 15 mL
3 Stain Solution A	Wright-Giemsa dye, 14 mL
4 Stain Solution B	Phosphate, 14 mL
5 Float	Φ 6 cm
6 Microcentrifuge Tubes with Agarose Gel	Low melting point agarose gel, 50 µL
7 Microcentrifuge Tubes with Agarose Gel (for Quality Control)	Low melting point agarose gel, 0.3 mL
8 Pretreated glass slide	Normal-melting agarose gel

The cut-off value for sperm DNA fragmentation evaluated by SCD was suggested by Dr. Budi Wiweko et. al. (Basic Clin Androl. 2017 Feb 21;27:1).

Limitation

- This kit is intended for testing human semen specimens only; product performance for other species has not been validated.
- For diagnostic purposes, the SCD assay and scoring of DNA fragmentation should be performed by certified personnels.
- The test results of SCD must be carefully evaluated and all other clinical-results related to the semen sample should be considered to assess male fertility.

Precision

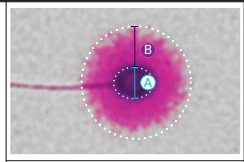
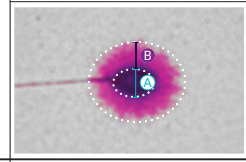
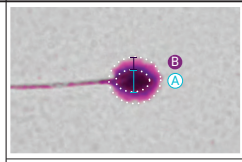
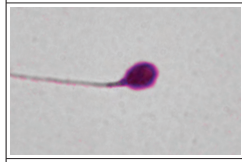
1. DFI value <20%: standard deviation (SD) ≦1
2. DFI value ≧20%: Coefficient of Variation (CV) ≦10%

Interference

The following substances have no significant interference on: white blood cells, pH value 6.4, 7.8, and 9.2.

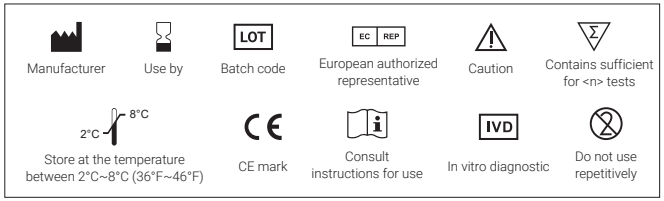
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user is established.

Classification of Sperm

Type	Image	Description
Unfragmented		**Large halo:** The halo width is similar or higher than minor diameter of the sperm core. Ⓑ ≥ Ⓐ
		**Medium halo:** The halo width is between large halo and small halo. Ⓐ/3 < Ⓑ < Ⓐ
Fragmented		**Small halo:** The halo width is similar or smaller than 1/3 minor diameter of the sperm core. Ⓑ ≤ Ⓐ/3
		**No halo**
		**Degraded:** Sperm shows no halo and presents an irregular core or a weakly stained head.

References

- Fernandez, J. L. et al. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl 24, 59-66 (2003).
- Fernandez, J. L. et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril 84, 833-842, doi:10.1016/j.fertnstert.2004.11.089 (2005).
- Wiweko B, et al. Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility. Basic Clin Androl. Feb 21;27:1. doi: 10.1186/s12610-016-0046-3.(2017)



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CE

Product Operation Video

Sample preparation

1 Dilute the semen sample with sperms extender of choice or **0.01M PBS** to a concentration of 5~10 × 10^6/mL.

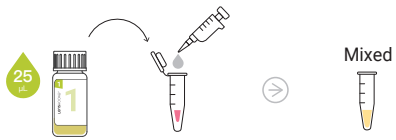
- Proceed the test immediately once you receive the sample.
- If Concentration is lower than 5~10x10^6/mL, Do not need to dilute. Can process SCD analysis directly.
- Please count 500 sperms at least to get the result. Therefore, if the concentration is lower than 0.1X10^6/mL, the sperm count might not enough.

2 Prepare **95~100°C hot water**. Then put the [6] **tube with agarose** into the floating plate and then put it into hot water for **1.5 mins** until the agarose fully melt.

- Make sure Agarose is melt or not : Flick the bottom of the microcentrifuge tube by finger to observe the flow of agarose.

3 -1 Take out the microcentrifuge tube, **flick the tube with agarose by finger** to make the remaining liquid drop down from the walls and caps to the bottom of the microcentrifuge tube. Or use mini centrifuge to centrifgue for 3~5sec.

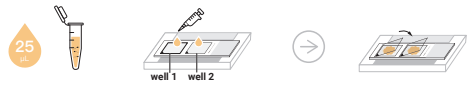
3 -2 Add **25μL of the [1] DNA Denaturant Reagent** to the **tube with agarose** and mix by pipetting. (3~5 times) until the color become **yellow**. Please avoid bubble.



4 Add **25μL of the diluted semen sample** from Step1 to the tube with agarose and mix by pipetting (3~5 times). Then move to Step5 immediately. Please avoid bubble.

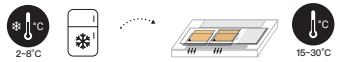
- In order to avoid Agarose gel temperature drop leading to solidification, Step3-1~4 operate-time do not over 2 mins.

5 -1 **Take 25uL** of the step 4 mixtures and apply it on test **well 1** and then get **another 25uL** drop to test **well 2**. After that, please cover up the glass coverslips separately. Handle it carefully to avoid the bubbles.



5 -2 Put glass slide into a Fridge, and incubate at 2~8°C for **5 minutes** to solidify the agarose.

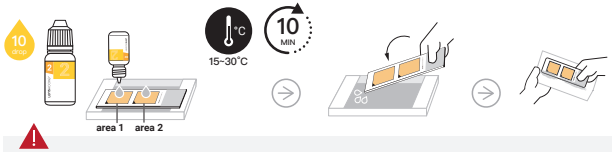
5 -3 Move the glass slide to room temperature (15~30°C) and **slide out** the coverslip gently.



- Do not use tools or pull up the coverslip by hand directly during the sliding process to avoid damaging the gel area.
- **After removing the coverslip, the gel should be Smooth and flat ○.**
- **If the gel is not flat, this glass slide is not qualified to do the test ×. Please follow the Step2-1 ~ 5-2 to prepare the glass slide again.**

Lysis

6 Place the slide on the slide staining tray and keep horizontality. Do not shake. After that, **drip 10 drops of [2] Lysis Solution individually to test area 1 and test area 2**. Please make sure if the Lysis solution fully covers the gel area. If yes, please leave the slide at room temperature for **10 minutes**. After that, please drain off the liquid and absorb the extra liquid.



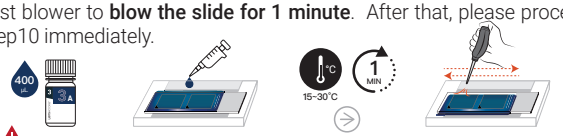
- It is important to avoid vibrate and shake during lysis step which will affect the halo image.
- Do Not shake or hit the slide when removing the lysis.

7 Place the slide on the slide staining tray and apply the **Distilled water**. Please make sure if the water **fully cover the slide** and the tray is on a flat table. If yes, please leave it at room temperature for **5 minutes**. After that please drain off the liquid and absorb the extra liquid.

8 Place the slide on the slide staining tray and apply the **95% Methanol** by pipette or dropper. Please make sure if the Methanol **fully cover the slide** and the tray is on a flat table. If yes, please leave it at room temperature for **1 minutes**. After that please drain off and absorb the extra liquid. **You can proceed the Step9 immediately without drying the slide.**

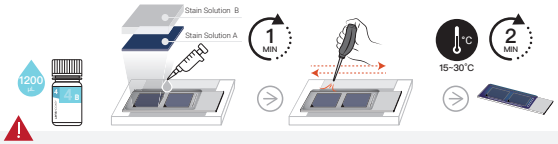
Staining

9 Upside down to mix the [3] **Stain Solution A** for 8~10 times, and **apply 400 μL [3] Stain Solution A** to the whole test area by pipette. Please make sure if the stain solution fully cover the slide. If yes, please use dust blower to **blow the slide for 1 minute**. After that, please proceed Step10 immediately.



- When you use the dust blower, we suggest to blow it from left to right and repeat 6~10 times.
- When you blow the slide, please separate the solution to let the glass contact with the air.
- Do not blow too much air to spill out the solution.

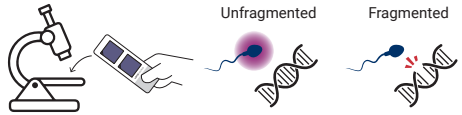
10 **Apply 1200 μL [4] Stain Solution B** to the whole test area by pipette. Please make sure the solution fully cover the slide. If yes, please use dust blower to **blow the slide for 1 minute**. After that, place the slide **at room temperature for 2 minutes**.



- When you use the dust blower, we suggest to blow it from left to right and repeat 6~10 times.
- Do not blow too much air to spill out the solution.

11 Rinse the slide glass clearly with **Distilled Water** for 20~30 seconds, then put at room temperature for drying.

Examine the sperms under bright field microscopy using 20x or 40x objective lens. Count 500 sperms per sample is recommended.



Calculate the percentage of sperms with fragmented DNA.
The formula used to calculate DNA fragmentation index (DFI) is :

$$\text{DFI} = \frac{\text{No. of sperms with fragmented DNA}}{\text{No. of sperms evaluated}} \times 100\%$$

Optional

If long-time storage of stained slide is needed, mount it with mounting medium for making permanent slides.

Quality Control

Positive control:

All sperms show no halo. Follow the procedure and skip Step6.

Negative control:

All sperms show halo. Follow the procedure and use 0.01M PBS to replace DNA denaturant reagent in Step3.