# QwikCheck® DFI KIT Instructions for Use

## Introduction and Intended Use

The QwikCheck DFI Kit is an in-vitro use, diagnostic kit for reporting sperm DNA Fragmentation Index (DFI) using the Sperm Chromatin Dispersion (SCD) method. This kit has been developed to simplify the laboratory process of creating slides to accurately assess sperm fragmentation based on WHO 5th or 6th methodology. The ratio of the size of the halos compared to the spermatozoa head can be assessed using a microscope or SQA Vision (with the WHO based Vision DFI counter). The QwikCheck DFI Kit is supplied with the necessary reagents to produce 10 test results (see table below). The QwikCheck DFI Kit is intended for use in clinical laboratories.

## Storage Conditions

The QwikCheck DFI Kit is supplied with the necessary reagents to produce 10 test results (see table below). Store the kit @ 2-25°C (36-77°F) for up to 1 year. Once opened, the kit can be stored @ 2-25°C (36-77°F) for 9 months (270 days). The expiration date assumes that the QwikCheck DFI Kit components are stored in their original containers and tightly capped to prevent evaporation. The components of the QwikCheck DFI Kit are stable and show no loss of expected performance characteristics after transport and storage over a period of 72 hours at a temperature range of 2-30°C (36°F to 86°F).

## Precautions:

- **Wear a laboratory coat and mask, and protective gloves.**
- **Use of a fume hood is recommended while preparing the slide (Steps 7 to 9).**
- **When first opening the bottles, remove the cap separator. Then re-cap the bottle while pressing down. This will puncture the bottle.**

## Materials and Methods for Use

| QwikCheck DFI Kit Components for 10 Tests | | | | |
|---|---|---|---|---|
| | **Reagents and Materials** | **#** | **Volume Supplied** | **Use** |
| 1 | Diluent (for sample see chart) | 1 | 10 mL / dropper bottle | Semen sample dilution |
| 2 | Low Melting Agarose (LMA) tubes | 10 | 0.06 mL in a 0.5 mL tube | To embed sperm |
| 3 | Agarose coated Slides | 10 | Slides (10) | To provide slides with the underlying matrix |
| 4 | Lys A Powder | 10 | 20mg/ 2 mL amber vial | Pre-measured powder. Mix with Lys A to obtain Lys Plus reagent |
| 5 | Lys A Solution | 1 | 10 mL / wide-mouth bottle | Solution to mix with Lys A powder to obtain Lys Plus reagent |
| 6 | Lys B Solution | 1 | 10 mL / dropper bottle | Sperm lysing |
| 7 | Fixative | 1 | 10 mL / dropper bottle | Sperm fixating |
| 8 | Giemsa A | 1 | 10 mL / dropper bottle | Sperm staining |
| 9 | Giemsa B | 1 | 10 mL / dropper bottle | Sperm staining |
| 10 | Coverslips 40mm x22mm | 20 | Coverslips (20) | Extra supplied in case of breakage during preparation |

## Checklist of equipment and materials required but not provided in the kit:

- SQA Vision / Microscope and Concentration Assessment Chamber/ Fume Hood/Refrigerator
- Two heating plates and 50ml glass beakers (one set to 90-100°C, and one 37°C) / Dry bath of both 70-80°C and 37°C
- Float, Staining Tray, and Filter Paper
- Centrifuge/ Centrifuge vials / Stopwatch and Precision Micropipette & Tips
- Distilled Water in Squeeze Bottles

## 1. Prepare Ahead:

- **Agarose Pre-Coated Glass Slides (10 min):** Bring to room temperature at least 10 minutes before use. Touch only the frosted region of the slide when removing from the box, and during the testing process.
- **Assess Sperm Concentration:** Check sperm concentration after sample liquefaction using the laboratory method. Do not pre-dilute the sample with any other reagent/media. **NOTE:** If α-chymotrypsin (QwikCheck Liquefaction Kit) is used for sample liquefaction, complete the DFI staining procedure within 1 hour of sample liquefaction.
- **Sample Dilution:** dilute the sample based on the native sperm concentration as shown in the following table.

| Sample Dilution Chart: Use only the QwikCheck sample diluent supplied in the kit | | | |
|---|---|---|---|
| **Concentration (M/ml)** | **Dilution Ratio** | **Volume** | |
| | | **Sample (µl)** | **Diluent (µl)** |
| <10 | Centrifuge | Decant the pellet | Resuspend pellet in 100 |
| 10 -30 | 1:2 | 100 | 100 |
| 31-50 | 1:4 | 100 | 300 |
| 51-70 | 1:6 | 100 | 500 |
| 71-100 | 1:7 | 100 | 600 |
| 101-150 | 1:10 | 100 | 900 |

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| | | | |
|---|---|---|---|
| Above 150 | 1:20 | 50 | 950 |

- **Make Lys Plus***: Add 1 mL of Lys A solution to one amber tube of Lys A powder. Close the lid and mix well by vortex or by manually rolling the tube between palms. Ensure that the powder has completely dissolved by aspirating with a micro-pipette or a plastic dropper.
  **NOTE: Use only one amber tube per test. Discard any leftovers and do not re-use or store them as the pH will change.**

## 2. Slide Preparation for Testing

1. **Melt Low Melting Agarose (@90-100°C):**
   Use 1 vial (tube) of agarose per test. To melt the agarose two methods are available: (1) HOT PLATE method: Fill a 50ml beaker with tap-water and place on a pre-heated to 95°C (for 5 minutes) hot plate. Place the low melting agarose vial in a float and keeping the top part of the vial out of the water, thoroughly melt the agarose for 30-45 seconds. Repeat if not melted/ (2) MICROWAVE method: Place the agarose on a float in a 50 ml beaker of tap water. Microwave on high (800 watts) for 45 seconds, if not melted, repeat for 30 seconds..

2. **Warm Low Melting Agarose (@37°C; ~5 min):**

   Transfer the agarose tube to a water bath (with a float)/dry bath maintained at 37°C and incubate for at least 5 minutes.

3. **Mix Diluted Semen and Melted Agarose (@37°C):**

   Add 40µL of the diluted semen to the melted agarose and mix by gentle pipetting, avoid creating bubbles.

4. **Prepare Agarose Coated Slide with the Semen/Agarose Mixture (@20-25°C):** Place two 20µL drops of the semen mixture, one drop below the other, in the center of the top of the clear (non frosted) section of the Agarose Coated Slide. Cover with a coverslip (22mmx40mm).

   **NOTE: This process is very temperature sensitive. Do not place the Agarose Coated Slide on metal surfaces.**

   **HINT: Place the coverslip slightly over the end of the Agarose Coated Slide so it can be easily removed later.**

5. **Solidify the Agarose (@ 2-8°C; 7 min):**

   Refrigerate the slide for 7 min @ 2-8°C (35.6-46.4F°) but do not place in the freezer.

6. **Remove the Coverslip (@20-25°C from this Step on):**

   Take the slide from the refrigerator after 7 min and gently remove the coverslip in a sliding motion, without breaking the agarose film embedding the spermatozoa. Place the slide onto a staining tray horizontally.

7. **Lys Plus* (5 min; 800 µl):**

   Pipette and place at least 800µl of freshly prepared Lys Plus on the slide and make sure the entire agarose area is covered. Wait 5 minutes.

8. **Decant Lys Plus* from the slide:**

   Gently tap the sides of the slide onto a staining tray or filter paper to remove the Lys Plus*. Wipe the **bottom** of the slide to remove any leftover Lys Plus solution.

   **NOTE: When wiping the remaining Lys Plus from the bottom of the slide, do not touch the agarose film area on top of the slide.**

9. **Lys B Sol (5 seconds maximum; ~4 drops):**

   Place at least 4 drops of Lys B solution on the agarose film area of the slide. Cover the whole agarose film area. Leave it on for **only 5 seconds.**

   **Decant Lys B from the slide:** Decant any residual Lys B and then wipe the **backside** of the slide, being careful not to touch the agarose area of the slide.

   **NOTE: Never exceed more than 10 seconds in this entire step.**

10. **Fixative (2 min; ~4 drops):**

    Place at least 4 drops of the FIXATIVE on the agarose film area of the slide and leave on for 2 minutes.

    **Decant Fixative from the slide:** Decant any residual Fixative and then wipe the **backside** of the slide, being careful not to touch the agarose area of the slide.

11. **Stain with Geimsa A solution (1 min; ~2 drops):**

    Place at least 2 drops of Geimsa A stain on the agarose film area of the slide. Leave it on for 1 minute **without decanting**.

12. **Stain with Geimsa B solution (2 min; ~4 drops):**

    Place at least 4 drops pf Geimsa B stain on the agarose film area of the slide. Make sure that the two solutions mix well and form an even layer, without dripping off the slide.

    Leave the solutions on the slide for 2 minutes until a **metallic sheen** can be seen forming on the top of the solution.

13. **Decant the Stain solutions and wash with Distilled Water (1-2 min maximum):**

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Tip the slide onto a staining tray or sink. Using distilled water in a squeeze bottle with a tip, gently wash the slide from above the agarose film area downward for 1-2 min at most. A light pink tinge will remain on the agarose film area of the slide.

14. **Air Dry the slide (5 min MAXIMUM):**

    Wipe the back side of the slide and Air Dry until visibly dry and no water droplets remain.

15. **Classify Halos:** Assess a minimum of 200 spermatozoa according to WHO 6th Edition Pg. 96., using the SQA-Vision DNA Fragmentation Assessment tool or under a microscope at 400X magnification.

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