



FiberProbes[®]

FSHD

REF FSH-HYB-002-IVD

CE **IVD**

Instructions For Use

IFU-M&S-10 version f-En (May 2019)



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1. BACKGROUND INFORMATION

FSHD (Facio-Scapulo-Humeral Muscular Dystrophy) is a genetic disease with autosomal dominant transmission defined by a progressive weakening and loss of skeletal muscles of the face (facio), the shoulder girdle (scapulo) and the upper arms (humeral). In 95 % of FSHD patients (also referred as FSHD1), a contracted 3.3kb-D4Z4 repeat array (1 to 10 copies) is present on a chromosome 4q35.2 subtelomere [1, 2] whereas the array contains between 11 and 150 repeats in the control population [3, 4]. In contrast, the FSHD2 form which is a rare form of FSHD is not linked to D4Z4 repeat array contraction. D4Z4 repeat arrays are also found on chromosome 10q26.1 subtelomeres, with no apparent relation to the disease. Chromosomes 4q and 10q subtelomeres may bear the haplotype A or B, which show several kilobases of divergent sequences. D4Z4 contraction is only pathogenic when it segregates in *cis* with the haplotype A on chromosome 4 [5-7]. The “CE-IVD FiberProbes® FSHD” enable the determination of the size of the D4Z4 repeat array and the A or B haplotype on both chromosomes 4q35.2 and 10q26.1 [8].

2. INTENDED USE

The “FiberProbes® FSHD” are designed for the diagnostic of FSHD1 by enabling the characterization of the 3.3kb-D4Z4 repeat-containing loci on chromosomes 4 and 10 by fluorescent hybridization on combed DNA extracted from blood samples and prepared according to the Molecular Combing procedures [8]. The “FiberProbes® FSHD” are intended to be used for *in vitro* diagnostic purpose.

In addition to the “FiberProbes® FSHD”, the FSHD1 diagnostic assay requires the use of the “FiberVision® Automated Scanner” for data acquisition and the “FiberStudio® FSHD Software” for the detection of the hybridized “FiberProbes® FSHD”, the analysis and the interpretation of the results (see the Instructions for use for FiberStudio® FSHD Software”).

3. PRINCIPLE OF THE FSHD1 DIAGNOSTIC ASSAY

1 - Extraction of genomic DNA



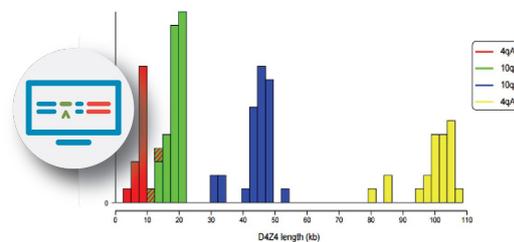
2 - Molecular Combing



3 - Hybridization with the FiberProbes® FSHD



4 - Scanning of FSHD1 signals with the FiberVision® Scanner



5 - Analysis with the FiberStudio® FSHD Software

Figure 1 - Workflow of the FSHD1 diagnostic assay on the Molecular Combing Platform

Molecular Combing allows the direct visualization and analysis of single DNA molecules [9, 10]. It enables DNA (extracted from fresh blood cells for *in vitro* analysis – Figure 1; step 1) to be stretched uniformly and irreversibly attached to a treated glass coverslip (step 2) according to the protocols for “DNA extraction and Molecular Combing” that are provided by Genomic Vision. The presence of specific genomic sequences (subtelomere regions of 4q35.2 and 10q26.1 in the test material) can be detected on stretched DNA by hybridization with labeled polynucleotides (“FiberProbes® FSHD”: step 3) and visualized by fluorescence microscopy (“FiberVision® Automated Scanner”: step 4) as it is described in the present Instructions for Use. The constant stretching factor allows the direct measurement of the probe length and the physical cartography of the region of interest. For the FSHD assay, the number of D4Z4 repeats in the test material is determined with the “FiberStudio® FSHD Software” (step 5) as described in the corresponding Instructions for Use provided by Genomic Vision.

4. PRODUCT CONTENT AND DESCRIPTION

“FiberProbes® FSHD” set: 5 x 20 µl per vial (2 tests per vial, 10 tests in total). One test is defined as sufficient for one hybridization experiment of combed DNA on coverslips (22 x 22 mm area).

The “FiberProbes® FSHD” contain fluorescein-, digoxigenin- or/and biotin-labeled polynucleotides that are intended to be used in combination with specific reagents (see Reagents and materials recommended but not provided – [6.1](#)) that allow their detection in green, blue, red and magenta fluorescent signals.

Labeled polynucleotides are premixed with hybridization buffer, and require the addition of deionized formamide just before use.

5. STORAGE AND HANDLING

Upon arrival, the product must be stored immediately between -25°C and -10°C protected from the light until the expiry date printed on the label. Improper storage of the product can destroy or impair the performance of the product and consequently the assay should not be performed from such reagent since it may affect the results.

Handle all reagents and slides in reduced light to prevent photobleaching of fluorophores.

Once thawed and prior to opening, the vials should be briefly centrifuged to ensure the contents are collected at the bottom of the vials.

Once opened, the remaining content of the vial can be frozen again and stored between -25°C and -10°C for up to 6 months.

Do not use the vials if:

- The package is damaged
- The product arrive thawed

All remaining reagent must be discarded in a biological waste bin.

6. WARNINGS AND PRECAUTION

For *in vitro* diagnostic use. For professional use only. Read the instructions carefully before use.

The analytical performances of the “FiberProbes® FSHD” were evaluated using coverslips with very long genomic DNA molecules linearly stretched at high density (Figure 2; picture 5) extracted from fresh blood samples. Using coverslips with genomic DNA at lower density (pictures 1-4) or short, wavy or tangled DNA molecules may affect the result of the *in vitro* diagnostic test.

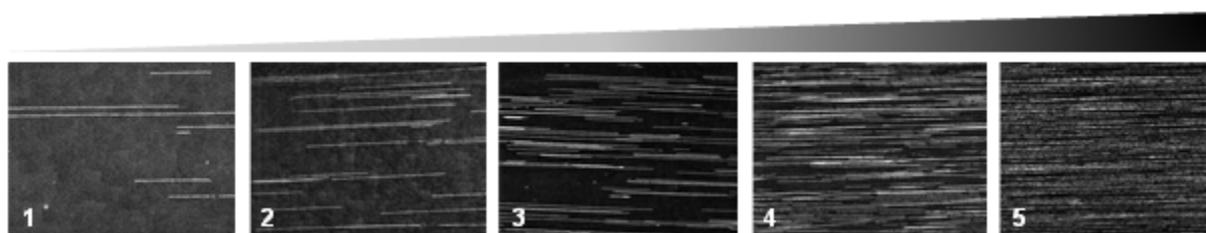


Figure 2 - Density of Combed Genomic DNA

Do not use the product after the expiry date.

As some substances contained in this product (in low concentrations and volumes) could be harmful to health, handle the reagents with care and wear appropriate personal protective equipment. See the corresponding Material Safety Data Sheet (MSDS) for safety information, the MSDS can be downloaded on the Genomic Vision website: www.genomicvision.com.

Refer to local regulations for appropriate elimination of waste.

6.1. Reagents and materials recommended but not provided for the hybridization and the detection of the “FiberProbes® FSHD”

IMPORTANT: Since the performance characteristics of the “FiberProbes® FSHD” have been evaluated and validated using the reagents and materials listed below, we recommend the use of these referenced reagents and materials for optimal diagnostic results.

The "FiberProbes® FSHD" were designed and their performance validated with the following accompanying reagents and materials for their detection as green, red, blue and magenta fluorescent signals:

- 0.5 mg Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescein (FITC) (Jackson ImmunoResearch Ref: 200-162-037). To be reconstituted (see §6.2 - Reagent preparation).
- 0.1 mg/ml BV480 Streptavidin (BD Biosciences, Ref: 564876). Ready-to-use.
- 0.5 mg Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin (Jackson ImmunoResearch Ref: 200-602-156). To be reconstituted (see §6.2 - Reagent preparation).

Note: Magenta fluorescent signal is the result of the double labeling of the corresponding probes that allows the detection of a co-localized blue and red signal with the above-mentioned reagents.

- CombiCoverslips™ IVD (Genomic Vision, Ref: COV-002-IVD).
- FiberVision® Automated Scanner (Genomic Vision, Ref: SCN-001)
- Sample holders for scanner (Genomic Vision, Ref: HLD-001)
- FiberStudio® FSHD Software IVD (Genomic Vision, Ref: FSE-FSHD-IVD)

Other reagents and materials are:

- Autoclaved distilled water
- Deionized formamide
- 20X SSC
- 70%, 90% and 100% ethanol
- BlockAid™ blocking solution
- Tween® 20
- 1X PBS
- Variable micropipette (1 µl - 200 µl)
- Tweezers
- Microscope slide
- Co-denaturation and hybridization instrument (e.g. Hybridizer, Dako)
- Ceramic coverslip tray
- Water bath with agitator, at +60°C
- Humidified chamber
- Incubator at +37°C
- 250 ml-beaker

6.2. Preparation of the reagents for the hybridization and the detection of the "FiberProbes® FSHD"

IMPORTANT: Use autoclaved distilled water for preparation of all stock and working solutions.

- **Hybridization Washing Buffer** (2X SSC solution): mix 100 ml of 20X SSC and 900 ml of distilled water. Store at room temperature.

Note: The buffer must be warmed at 60°C in waterbath with agitation prior to use.

- **Detection Washing Buffer** (2X SSC/1% Tween): Mix 100 ml of 20X SSC and 10 ml of Tween® 20 with 890 ml of distilled water. To be prepared extemporaneously.
- Reconstitute the lyophilized reagents with autoclaved distilled water as indicated in the table below:

	Quantity (mg)	Volume of distilled water to be added (ml)	Final concentration (mg/ml)
Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescein (FITC)	0.5	0.350	1.43
Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin	0.5	0.350	1.43

- Prepare extemporaneously the **Detection Solution**, containing the mix of reagents needed for the detection of hybridized probes as follows (quantities indicated for 1 test):

	Volume (µl)
- 1.43 mg/ml Cy™3 IgG Fraction Monoclonal Mouse Anti-Fluorescein (FITC)	0.8
- 0.1 mg/ml BV480 Streptavidin	0.8
- 1.43 mg/ml Alexa Fluor® 647 IgG Fraction Monoclonal Mouse Anti-Digoxin	0.8
- BlockAid™ Blocking Solution	17.6

7. OVERALL ASSAY PROCEDURE

7.1. Hybridization of the FiberProbes® FSHD on combed DNA

IMPORTANT: All the steps where formamide is used must be performed under a fume hood.

1. If the combed coverslips were stored at -20°C, Take them out and leave them 10 min at room temperature on a ceramic coverslip tray.
2. Dehydrate the combed DNA coverslips by dipping the ceramic coverslip tray 1 min at room temperature in successive baths of 70%, 90% and 100% ethanol.
3. Air dry the coverslips at room temperature for at least 10 min protecting from light.
4. Thaw the vial containing the “FiberProbes® FSHD” during 10 min at ambient temperature, protecting from light.

Attention, each tube contains the necessary volume for 2 tests.

5. For one test, transfer 10 µl of the “FiberProbes® FSHD” in a new microcentrifuge tube and add 10 µl of deionized formamide to the “FiberProbes® FSHD”.
6. Mix well and incubate at 37°C for 30 min.
7. Pipette 20 µl of the probes/formamide mix and pour cautiously the drop on a clean microscope slide, taking care not to form any air bubble.
8. Avoiding trapped bubbles, use tweezers to very carefully set the coverslip on top of the drop of hybridization solution, engraved number facing down (in contact with the drop). Once mounted, do not move or push the coverslip in order to avoid scratches.

Note: Make sure to identify which side of the coverslip is in contact with the hybridization solution to avoid confusion in the subsequent immunodetection steps. Engraved number is proposed to facilitate this identification.

Note: From this step on, coverslips must be protect from light at all times.

9. Co-denature the combed DNA on the coverslip and the probes for 5 min at 90°C in the humidified chamber of a hands-free co-denaturation and hybridization instrument (ie. Dako hybridizer).
10. Incubate for 16-20 hrs at 37°C in the humidified chamber of the co-denaturation and hybridization instrument.

The next day:

11. Incubate the Hybridization Washing Buffer in a waterbath at 60°C with agitation during approximately 1h, in order to pre-warm it.
12. Remove the coverslip from the microscope slide and place it on a ceramic coverslip tray. Insert the ceramic tray in a 250 mL-beaker containing the pre-warmed (60°C) Hybridization Washing Buffer, in sufficient volume to immerse the coverslip entirely.

***Note:** If the coverslip is stuck on the slide, add a drop of Hybridization Washing Buffer around the coverslip and wait until the coverslips floats or dip the slide for a few seconds in warm Hybridization Washing Buffer. To remove the coverslip, slide it gently until one corner comes out of the slide, then gently lift it using tweezers, to avoid making scratches.*

13. Wash the coverslip three times in pre-warmed Hybridization Washing Buffer for 5 min each at 60°C in the waterbath without agitation, replacing the Hybridization Washing Buffer each time.

***Note:** The coverslips must be totally immersed in the solution. Do **not** let the coverslip dry between the washing steps, as this could reduce significantly the performance of the subsequent immunodetection step.*

7.2. Detection of the FiberProbes® FSHD

1. Pipette 20 µl of the Detection Solution on a microscope slide and set the coverslip on the drop of Detection Solution.

***Note:** Make sure to place the hybridized side of the coverslip in contact with the Detection Solution.*

2. Incubate the slide for 20 min in a humidified chamber at 37 °C.
3. Remove the coverslip from the microscope slide and place it on a ceramic coverslip tray in a 250 ml-beaker containing the Detection Washing Buffer, in sufficient volume for the coverslip to be completely immersed in the solution.
4. Wash the coverslip three times in the Detection Washing Buffer for 3 min each at room temperature with gentle agitation (100 rpm on an orbital agitator). Replace the Detection Washing Buffer each time.
5. Wash the coverslip in 1xPBS for 3 min at room temperature with gentle agitation (100 rpm on an orbital agitator).

6. Dehydrate the coverslip by dipping the ceramic coverslip tray 1 min in successive baths of 70%, 90% and 100% ethanol without agitation, at ambient temperature.
7. Air dry the coverslip at room temperature for at least 10 min protecting from light.
8. From this step, the coverslip can be stored at 4°C protected from light until observation using an epifluorescence microscope.

7.3. Visualization of the fluorescent signals

For visualization of the fluorescent probes, the hybridized coverslips should be scanned using a fluorescent microscope equipped with a 40X objective and the following filters:

Fluorophore	Excitation peak (nm)	Emission peak (nm)
Cy3	543 ± 30 nm	588 ± 55 nm
BV 480	434 ± 17 nm	515 ± 23 nm
AF647	640 ± 14 nm	700 ± 70 nm

For the FSHD1 diagnostic assay, the hybridized coverslips must be scanned with the FiberVision® Automated Scanner, an automated image scanning system developed by Genomic Vision, and analyzed using the “FiberStudio® FSHD Software”.

The hybridization signals appear as multicolor fluorescent signal arrays. The 3.3kb-D4Z4 repeats appear in green, while signals which distinguish 4q and 10q chromosomes and qA and qB haplotypes, appear in red, blue and magenta with a specific size, spacing and order (as indicated in the Figure 3). The different fluorescent signals that might be detected are as follows:

- Chromosome 4q35.2 is identified by a centromeric 18kb-red signal, a ~1.5 kb Dux4 green signal, a 10kb-magenta signal, a 5kb space and a 20kb-blue signal;
- Chromosome 10q26.1 is identified by a centromeric 15kb-blue signal, a 11kb space, a 10-kb magenta signal, a 5kb space and a 20kb-blue signal;
- Haplotype qA appears as a 2 telomeric 6kb red signals
- Haplotype qB appears as a 2 telomeric 6kb blue and red signals.
- An additional genomic region on chromosome 3, resulting in a sequence homology unrelated to FSHD1, is also detected as 2 blue fluorescent signals surrounded by red and magenta signals.

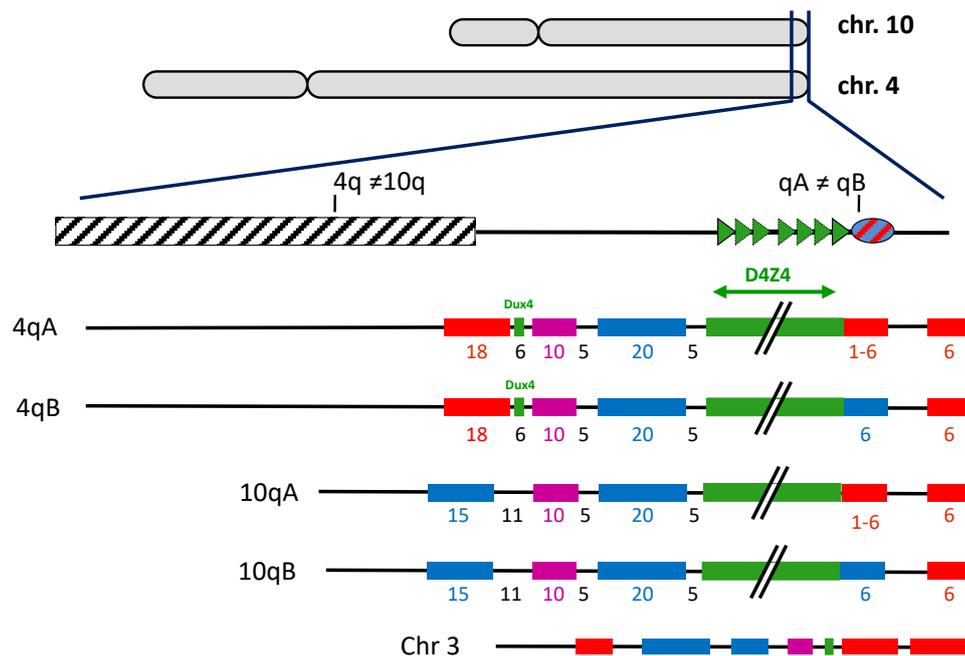


Figure 3 - FiberProbes® FSHD Genomic Morse Code (GMC) Design

Note: The colors showed in Figure 3 are artificial colors visualized in the FiberStudio® FSHD software, not necessarily corresponding to the colors produced by the wavelength emitted by fluorescent antibodies.

For the diagnostic of FSHD1, the detection, classification and the measurement of the different fluorescent FSHD signals (especially the length of D4Z4 repeat array) can be performed using the “FiberStudio® FSHD software”. Please refer to the Instructions for Use of the “FiberStudio® FSHD software” or contact the manufacturer for additional information.

The performances of the FSHD test are guaranteed only using the FiberProbes® FSHD combined with the FiberStudio® FSHD software, in its validated CE-IVD version.

8. INTERFERENCE

The FiberProbes® FSHD generate an additional signal on Chromosome 3 (indicated as Chr 3 in the figure 3 above) but does not interfere with the results since its specific fluorescent pattern allows its identification. These signals are not included in the analysis and in the diagnostic result.

9. RECOMMENDATIONS AND LIMITATIONS

The FiberProbes® FSHD are designed for in vitro diagnostic use (according to the EU directive 98/79/EC) and have to be analyzed with the FiberStudio® FSHD Software, in its validated CE-IVD version. Interpretation of results must be made within the context of the patient's clinical history by a qualified pathologist. A medical decision cannot be made based on the results of this assay alone. The FiberProbes® FSHD cannot be used for pre-natal diagnostic or for other diagnostic use (e.g. other muscular disorders). Genomic Vision does not assume any responsibility for improper application of this product.

10. TRAINING

Training is mandatory for performing this assay. Genomic Vision will provide training in specimen preparation, assay procedure, and interpretation of Molecular Combing testing of FSHD for inexperienced users. It is also recommended that a laboratory that has previously received training but now has new personnel performing the assay request training for the new users.

11. PERFORMANCE CHARACTERISTICS

11.1. Accuracy

The performances of the FiberStudio FSHD CE-IVD software have been evaluated in comparison with the gold standard, the Southern Blot (ou PFGE). This performance study has been carried out with an external laboratory on 80 samples from patients.

This sample plan insures a statistical power ($1-\beta$) of 90% for this correlation study.

The percentages of samples which have a number of D4Z4 repeated units similar by Southern Blot (ou PFGE) and by Molecular Combing FSHD test using the FiberStudio FSHD CE-IVD software, are:

- 83 % with ± 1 D4Z4 repeated units
- 97 % with ± 2 D4Z4 repeated units

Moreover, for 31 alleles with short 4qA which size is within the "grey" zone for the FSHD genetic disorder (between 7 to 13 D4Z4 repeat units), the percentages are equivalent at the results obtained for all samples of the study:

- 81 % with ± 1 D4Z4 repeated units
- 94 % with ± 2 D4Z4 repeated units

11.2. Measuring range of the assay

The 80 samples included in the performance study, are distributed within a measuring range from 2 to 71 D4Z4 repeated units.

However, we do not claim a restriction of the measuring range for values above 71, as the diagnostic risk is negligible for these types of measurements.

For values below 2 D4Z4 repeated units, the size of ROI is close to the detection limit of the software. ROI are however present and can be detected manually by the operator. The analysis can then be normally executed by the software.

11.3. Repeatability/Reproducibility

A study was performed on patient samples and GM17939 cell line, derived from a patient affected by FSHD1. Samples were analyzed by 2 independent laboratories:

- 5 samples were tested in the same conditions, processed by the same operator at different dates
- 10 samples were tested once into 2 different laboratories and processed by 2 different operators

The results show an equivalence between the replicates and operators with a maximum of 2 repeated units' difference.

12. ORDERING INFORMATION AND RELATED PRODUCTS

Product	Contents	Reference
FiberProbes® FSHD CE-IVD	10 hybridizations	FSH-HYB-002-IVD
FiberStudio® FSHD Software CE-IVD annual license, support & update	pack 1 user	FSE-FSHD-IVD-P1
	pack 2-3 users	FSE-FSHD-IVD-P2
FiberPrep® DNA Extraction Kit	100 extractions	EXT-001
	10 extractions	EXT-001-10
FiberComb® MCS CE-IVD	Combing Device	MCS-001

Product	Contents	Reference
CombiCoverslips™ CE-IVD	Box of 50 units	COV-002-IVD
Disposable Reservoirs CE-IVD	Pack of 10 units	RES-001
Reservoir Supports	Provided by 2 units	SUP-001
Reservoir Bench Holder	Holder with 10 positions	POR-001
Coverslip Clip Holder	Holder with 2 positions	CLI-001
FiberVision® Scanner	Automated Scanner	SCN-001
Sample Holders for Scanner	Pack of 25 units	HLD-001

To place an order, please contact us at sales@genomicvision.com
or visit our website <http://www.genomicvision.com/gv-store/>

13. REFERENCES

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Our experts are available to answer your questions

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Patent

This product or the use of this product is subject to proprietary rights (EP2007/059299, IB2009/007197). The Molecular Combing technology and products are covered by patents (FR2716206, FR 2716263, FR 2737574, FR 2755149) owned by Genomic Vision S.A.

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BlockAid is a trademark of Molecular Probes, Inc.

Cy is a trademark of GE Healthcare Bio-Sciences Ltd

Alexa Fluor is a trademark of Life Technologies Corporation.

Tween is a registered trademark of Croda International PLC



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