Adellgene®

Fragile X

INSTRUCTIONS FOR USE

Kit to determine the number of CGG triplet repeats in the *FMR1* gene using fragment analysis technology

CE

Rev. 02 - 2022/05/12

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www.bdrdiagnostics.com

Product codes:	UDI-DI	Store:
AD-FMR1-25	8437016942468	from -18°C to -30°C
AD-FMR1-100	8437016942475	

Adellgene®

Fragile X

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1 – Information for safety

Please, read completely these instructions for use and apply them during the use of the current IVD kit.

The IVD kit shall be used by experts with strong experience in DNA analysis and interpretation of genetic results.

Consult the manufacturer if there are doubts involving the description of the assay method. Contact by phone +34 976 094 603 or email address *customersupport@bdrdiagnostics.com*.

The IVD kit has a limited shelf life. Keep sure that the shelf life is not expired before using the kit. Reagents from a kit beyond its expiry date might be degraded, which could impair results. Discard the expired reagents following applicable regulations.

The contamination of the sample or the reagent can render incorrect results. Be careful during the DNA extraction and sample and reagents manipulation.

This kit can be damaged during transport or storage. Do not use the kit in case of suspected damage during transport. Follow carefully the storage conditions described in the label and the IFU.

Ensure that the waste is managed according to local regulations. An incorrect waste management can result in environmental contamination.

Toxicological properties of the kit have not been studied in depth, so it is recommended to avoid contact with skin and mucous membranes. Do not ingest. Manual Safety Data Sheets (MSDS) are available for the customers by request.

Ensure that this kit is adequate for the requested analysis by the clinician.

2 – Intended Use

Adellgene® Fragile X is a semi-automated *in vitro* diagnostic kit designed for use in clinical laboratories for the amplification and detection of CGG triplet repeats (cytosine-guanine-guanine) in the 5' untranslated region of gene for fragile X mental retardation ("Fragile X mental retardation-1": *FMR1*). It aims to aid diagnosis of clinical disease associated with Fragile X syndrome (e.g.: mental retardation, primary ovarian failure, tremors / ataxia, etc...).

The technology is based on the polymerase chain reaction (PCR) amplification of genomic DNA extracted from peripheral blood, followed by fluorescence analysis of the size of the PCR fragments obtained by genetic analyser.

Patients who can benefit from this determination are those referred by a specialist. The intended user of the kit is technical personnel trained to carry out the protocol and the interpretation of results described in this document.

3 – Summary and explanation

Fragile X Syndrome (FXS, OMIM #300624) is an X-linked disease that is mainly based on the genomic expansion of the CGG (cytosine-guanine-guanine) triplet of nucleotides, located in the 5' untranslated region of the *FMR1* gene (Fragile X Mental Retardation 1) on chromosome Xq27, and aberrant methylation status of the promoter of the gene (Fig. 1) [1].

Three categories can be established based on the number of triplet repeats:

- Healthy alleles: up to 45 or 55 CGG repeats without methylation of the promoter of the gene [2].
- Premutation alleles: from 45 or 55 to 200 CGG repeats, usually without aberrant methylation. In this case, individuals are asymptomatic for disorders associated with FXS but can be associated with two clinical disorders: Fragile X Syndrome-Associated Tremor/Ataxia (FXTAS) and Fragile X-Associated with Primary Ovarian Insufficiency (FXPOI) whose severity depends on the methylation state of the promoter [3, 4]. It is estimated that the prevalence in the general population is 1 in every 110-270 females and 1 in every 250-810 males [1]. Moreover, premutation alleles are unstable and may increase in size during maternal transmission to the offspring, resulting in a full mutation [5].
- Expanded alleles: over 200 CGG repeats, with aberrant methylation of the promoter of the gene. This genotype leads to the elimination of the gene expression in the human brain, which is associated with mental retardation, autism, and mental and emotional changes. Patients with expanded alleles show a striking phenotype consisting of large ears and prominent jaw [1]. The severity of cognitive impairment in patients with FXS is not associated with the magnitude of the full mutation but varies with the state of methylation [6].

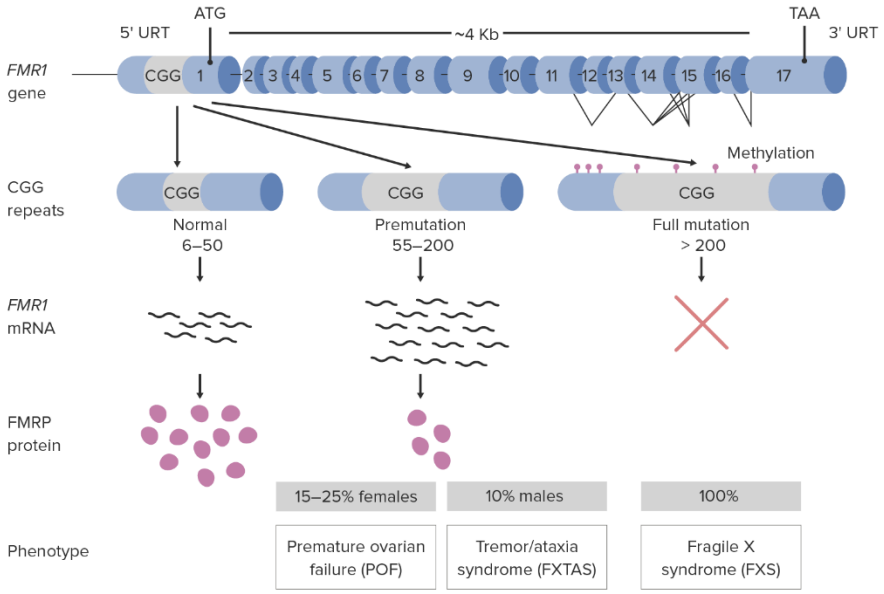


Figure 1. Depiction of the types of FMR1 gene and their production depending on the degree of mutation. Extracted from [7].

Many *FMR1* alleles contain interspersed AGG sequences between the CGG repetitions. It is thought that these inserts may confer stability to the DNA and reduce the risk of triplet repeats expansion in the next generation. Therefore, the likelihood of CGG repetitions expansion in the offspring is higher when the mother does not present AGG insertions in the *FMR1* allele [5, 8].

One of the most common methods for molecular diagnosis of Fragile X Syndrome is the Southern blot of genomic DNA digested with the restriction endonucleases EcoRI and EagI and hybridized with a specific probe [9, 10]. Although this method allows to quantify the number of CGG triplet repeats and determine the methylation status in some cases, it has many inconveniences. It requires a large amount of DNA input and has labor intensive and time-consuming protocols, making it unsuitable for high throughputs. Moreover, it does not

allow to determine the methylation state in most samples of DNA extracted from chorionic villi (CVS) due to its low sensitivity and because the methylation process is only fully completed beyond the tenth week of pregnancy [11].

Nowadays the PCR is the most extended technology, based on the specific amplification of the affected region of the gene. However, this process can be impaired due to the presence of a highly GC-rich sequence within the *FMR1* gene. To overcome these drawbacks, several variations in the amplification process have been described. These include the use of osmotic adjuvants, modified nucleotides and setting specific conditions of the PCR process [12].

4 – Procedure principles

The method used by the Adellgene® Fragile X kit is based on specific amplification of the the 5' untranslated region of the *FMR1* gene, which contains the CGG triplet repeats, using genomic DNA.

The kit includes various primers, one of which is marked with a fluorophore (FAM) for its subsequent detection in a genetic analyser. Later, the size of the PCR fragments is converted into the number of CGG repeats, using mobility and size correction factors.

5 – Kit contents

➔ **Reference AD-FMR1-25 (25 tests):**

- AD-FMR1-PM: Primer Mix. 1 vial, 25 µL
- AD-FMR1-POM: Polymerase Mix. 1 vial, 12.5 µL
- AD-FMR1-GC: GC-Rich Buffer. 1 vial, 300 µL
- AD-FMR1-SL: ROX 1000 Size Ladder. 1 vial, 50 µL

➔ **Reference AD-FMR1-100 (100 tests):**

- AD-FMR1-PML: Primer Mix. 1 vial, 100 µL
- AD-FMR1-POML: Polymerase Mix. 1 vial, 50 µL
- AD-FMR1-GCL: GC-Rich Buffer. 1 vial, 1.2 mL
- AD-FMR1-SLL: ROX 1000 Size Ladder, 1 vial, 200 µL

6 – Kit storage

All reagents must be stored between -30°C and -18°C. At these temperatures they are stable up to their expiry date.

Do not freeze/thaw the Primer Mix vials (AD-FMR1-PM/AD-FMR1-PML) and the Polymerase Mix (AD-FMR1-POM/AD-FMR1-POML) more than two times as this could reduce the test's sensitivity. If tests are to be carried out with few samples, it is recommended to use aliquots of the reagents.

Due to the photosensitive nature of the reagent, avoid continuous exposure to light.

7 – Materials required but not supplied

➔ Reagents

- Isolation and purification reagents for DNA extraction. DNA can be extracted using any laboratory-validated extraction system that allows the recommended concentrations and levels of purity for this test to be obtained (consult the Specific operation data section)
- DNAse-free distilled water
- POP-7 polymer (ABI, Ref: 4363785 or equivalent)
- Highly deionized (Hi-Di) formamide (ABI, ref.: 4311320)
- Calibrators for fluorophores FAM and ROX (ABI, ref.: 4345827)

➔ Equipment

- General laboratory equipment dedicated to PCR techniques
- Thermal cycler
- Genetic analyser running POP-7 polymer (e.g.: ABI: 3130/3130xl, 3730/3730xl or 3500/3500xl)
- PCR plate/tube centrifuge
- Vortex mixer
- Pipettes (P1000, P200, P20 and P2) and specific filter tips for each pipette
- PCR plates/tubes with their corresponding covers/caps

8 – Sample collection and preparation

Samples must be collected in line with the instructions for use of the collection device (not included with the kit) and in accordance with national and international guidelines.

This kit should only be used with whole blood samples preserved with anticoagulant agents such as EDTA or citrate. Heparin could interfere with the PCR process and should be avoided.

Do not use hyperlipemic, haemolysed, icteric or proteinemia samples.

The technique is compatible with several conventional DNA extraction systems. Before delivering results with diagnostic purposes, perform a validation test with the extraction method used.

CAUTION!

All biological and blood samples must be treated as potentially infectious. When handling them, take the corresponding basic and universal precautions.

9 – Usage procedure

➔ DNA extraction

This kit is compatible with any standard DNA extraction method from whole blood preserved with EDTA or citrate. However, before proceeding with the general protocol, it is recommended to perform a test on the extraction method used to assess the concentration values (absorbance of 260 nm) and purity values (ratio of absorbance 260/280 nm).

The recommended amount of DNA is 20 – 80 ng (e.g.: 2 µl of DNA at a concentration of 10-40 ng/µl), with a OD260/280 ratio > 1.7. The DNA obtained can be stored at -20°C until used.

➔ Preparation and conditions of the PCR reaction

WARNING!

- Allow all kit components to fully thaw before using.
- Once thawed, vortex and centrifuge to ensure the entire volume of reagent is settled at the bottom of the vial.
- Always work on ice or a cold block, minimising the reaction preparation time as far as possible.
- Establish a pre- and post-PCR work area to prevent contamination phenomena.
- Only use filter tips and autoclaved or sterile tubes.
- Always wear a laboratory coat and gloves.

4. Configure the thermal cycler with the following amplification program:

	Number of cycles	Temperature (°C)	Time (mm:ss)
DENATURATION	1 hold	98	05:00
CYCLES	10 cycles	97	00:35
		62	00:35
		68	04:00
CYCLES	20 cycles	97	00:35
		62	00:35
		68	04:00 + 00:20 each additional cycle
FINAL EXTENSION	1 hold	72	10:00
COOLING	1 hold	4	∞

5. Prepare the following reaction mixture for n+1 samples:

Reagent	Volume per sample (µL)
Primer Mix (AD-FMR1-PM / AD-FMR1/PML)	1.0
GC-Rich Buffer (AD-FMR1-GC / AD-FMR1/GCL)	12.0
Polymerase Mix (AD-FMR1-POM / AD-FMR1-POML)	0.5
Distilled water	4.5
Total volume per reaction	**18.0**

6. Gently mix the reaction mixture by pippeting 3-5 times to ensure a complete homogenisation before distributing it into the PCR tubes.
7. Dispense 18 µL of this reaction mix into each well or tube.
8. Add 2 µL of the appropriate DNA sample (10-40 ng/µL), or water in the case of contamination control well, to reach a final volume of 20 µL.
9. Seal the plate (or tubes) with coverslips and perform a brief centrifugation to remove any bubbles. Ensure that all the volume is settled to the bottom of the tube.
10. Place the plate or tubes in the thermal cycler and run the PCR program.
11. After the run, continue with sample preparation for the capillary electrophoresis or store the PCR product at -20ºC.

➔ Sample preparation for capillary electrophoresis

1. Thaw formamide and ROX™ 1000 size standard.
2. Vortex and spin the tubes before use.
3. Prepare the following mix adding the components specified in the table below for n+1 samples:

Reagent	Volume per sample (µL)
Hi-Di™ Formamide	8.0
ROX 1000 size ladder	1.0
Total volume per reaction (µL)	9.0

12. Mix the solution and spin down to collect.
13. Dispense 9 µL of the solution to each well of the capillary electrophoresis plate.
14. Transfer 1 µL of the PCR product to the plate and mix by pipetting.
15. Seal the plate and centrifuge to remove bubbles. Transfer the plate to the thermal cycler.
16. Denature the DNA following the program below:

	Number of cycles	Temperature (ºC)	Time (mm:ss)
DENATURATION	1 hold	95	02:00
COOLING	1 hold	4	∞

17. After the run, transfer the plate to ice and protect it from light until the inyection on the genetic analyser.

➔ Module of the genetic analyser

The following module is recommended for the corresponding genetic analyser:

Parameters	Genetic analyser		
	3130/3130xl	3730xl	3500/3500xl
Oven temperature	60°C	63°C	60°C
Current stability	5.0 µA	5.0 µA	5.0 µA
Pre-run voltage	15.0 kV	15.0 kV	15.0 kV
Pre-run time	180 s	180 s	180 s
Injection voltage	2.5 kV	2.5 kV	2.5 kV
Injection time	20 s	20 s	20 s
Voltage number of steps	20 nK	20 nK	40 nK
Voltage step interval	15 s	15 s	15 s
Data delay time	60 s	60 s	1 s
Run voltage	15.0 kV	15.0 kV	8.5 kV
Run time	2400 s	4000 s	4000 s

NOTE: The module of the analyser may differ between instruments, and it should be validated by the user.

10 – Results and interpretation

The Adellgene® Fragile X kit is a quantitative technique to determine the number of CGG triplet repeats in the 5'UTR of the *FMR1* gene. The kit allows the quantification of expansions up to 200 repeats.

The determination of these alleles is considered difficult because of the high concentration of GCs, which creates a strong secondary structure that prevents replication of this region even with the use of Taq polymerase at high temperatures. The Adellgene® Fragile X kit includes a series of reagents that allow this region to be amplified and the number of repeats to be determined, enabling the differentiation between non-expanded, premutation and expanded individuals.

The use of a molecular size marker with known fragment sizes allows the sequencer's software to calculate the size of the amplified product directly, through the determination of each peak's size.

➔ Standardisation process

To standardise the fragments' mobility in the particular conditions of electrophoresis in which the experiment is carried out (polymer, genetic analyser, etc.), two correction factors are used in the calculation of number of CGG repeats: the size correction factor (C_0) and the specific mobility correction factor (m_0).

C_0 and m_0 values can be calculated using a pooled DNA control (correction factor reagent). This control is a mixture of several PCR products including *FMR1* alleles with a known number

of triplet repeats, and ranging through the entire peaks' size range (<200 repetitions). C_0 and m_0 values are determined from a linear fit of the number of repeats and fragment size for these alleles.

The correction factor reagent can be prepared by mixing equal volumes of PCR amplicons. See references commercially available below.

Catalog number	CGG repeats by Adellgene® Fragile X kit.
NA20235	29, 45
NA20236	31, 54
NA06891	118
NA20239	20, 199

Table 1 Reference DNA templates composing a correction factor reagent. For more information, see https://www.coriell.org/

To calculate the values of the correction factors to be used in the experiment, proceed as follows:

1. Analyse the correction factor reagent in the run and calculate the peaks' size for each of the *FMR1* alleles. It is recommended to determine the average size in base pairs of the alleles from two separate runs.
2. In Excel or similar program, create a table with the previous data and the number of triplet repeats of each allele and insert a scatter chart. Perform a linear regression obtaining the corresponding equation to the linear fit (Figure 2).

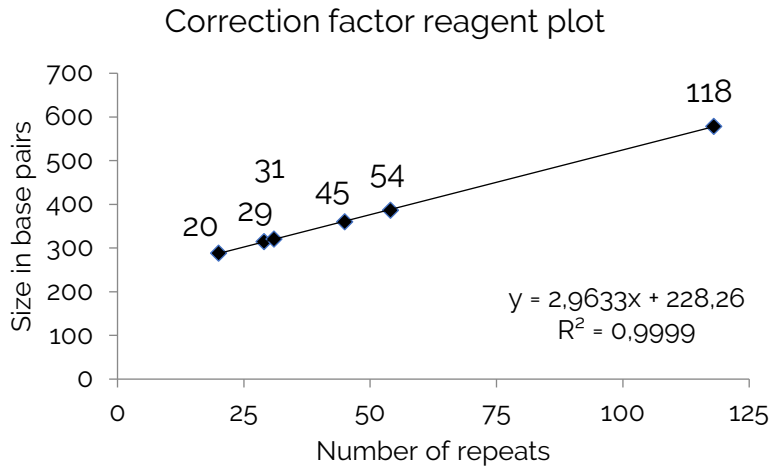


Figure 2. Correction factor reagent plot

From the equation of this regression fit, the correction factors are determined: C_0 corresponds to the intercept of the linear fit, whereas m_0 corresponds to the slope of the line. In the graph shown, C_0 = 228.26 and m_0 = 2.9633.

➔ Electropherograms interpretation

To determine the number of CGG repeats, identify gene-specific full length product peaks (corresponding to the full size of the alleles) in the electropherograms. Deselect the CGG ladder peaks so only the gene-specific amplification product will stay selected. See Figure 3 for an example.

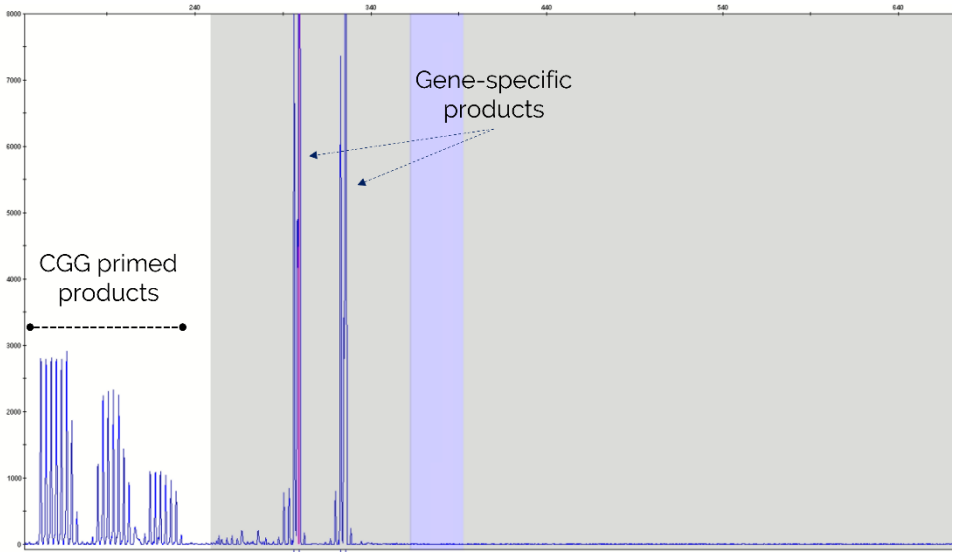
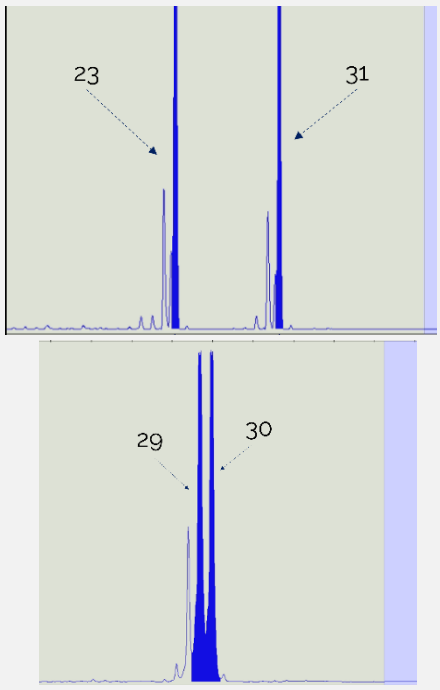


Figure 3. Electropherogram identifying the gene-specific products.

Figure 4 shows guidelines to identify main target peaks among gene-specific product peaks.

Allele ranges	Guidelines to select main peaks	Examples
Normal and intermediate alleles	Select the highest peak of a single allele group. In the case of heterozygous alleles with one CGG repeat difference (e.g. 29/30), two allele groups may not be fully-separated. In this case the two highest peaks should be selected.	

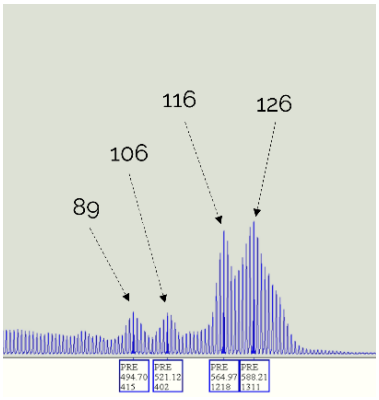
Premutation alleles	Select the highest peak of the peak population. In the case of alleles with several peak populations, select the highest peak of each group.	

Figure 4. Identifying the main peaks in the electropherograms.

➔ Calculation of the number of CGG repeats

To calculate the number of repeats corresponding to each allele, the equation given next must be applied:

$$No.\,of\ triplet\ repeats = \frac{(Peak\ size - C_0)}{m_0}$$

Size of the peak (in base pairs)

C_0 – Size correction factor

m_0 – Mobility correction factor

Correction factors can be determined following the procedure described in "Standardisation process" section. These values may vary laboratory from laboratory, consider using positive controls in order to verify the accuracy of your number of repeats estimation when using the kit for the first time.

➔ Electropherogram results

Examples of electropherogram results obtained with this kit are shown in the following images: healthy heterozygous female (Figure 5), heterozygous female with a healthy allele and another premutation or expanded allele (Figures 6-7) and an expanded male (Figure 8).

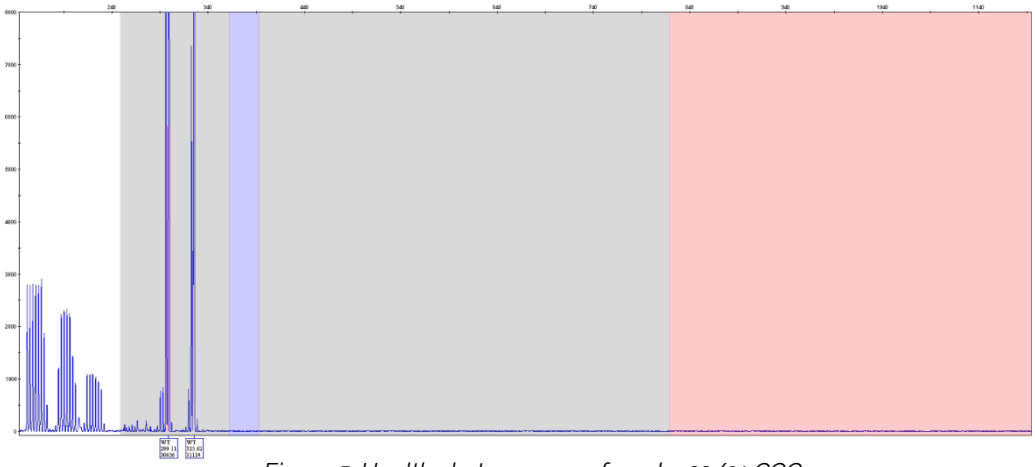


Figure 5. Healthy heterozygous female, 22/31 CGG.

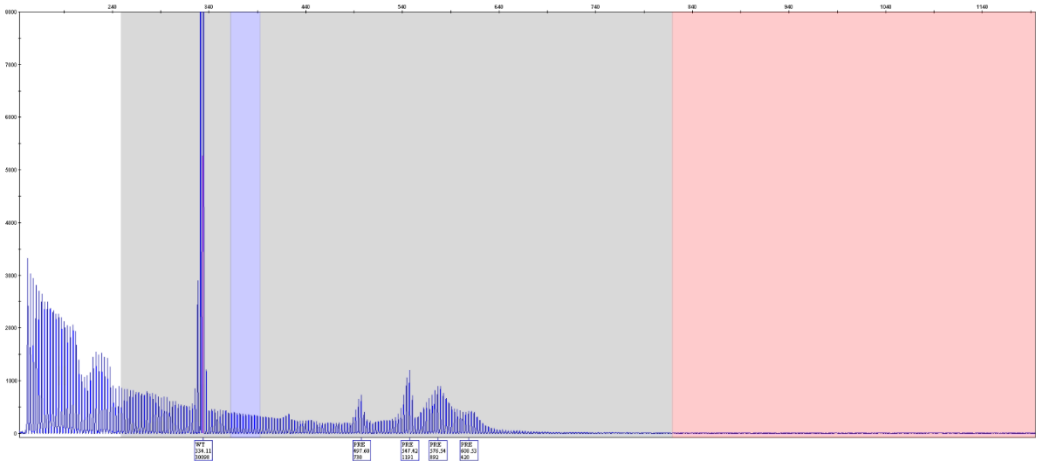


Figure 6. Heterozygous female with a premutation allele in mosaic, 35/89, 106, 117, 128 CGG

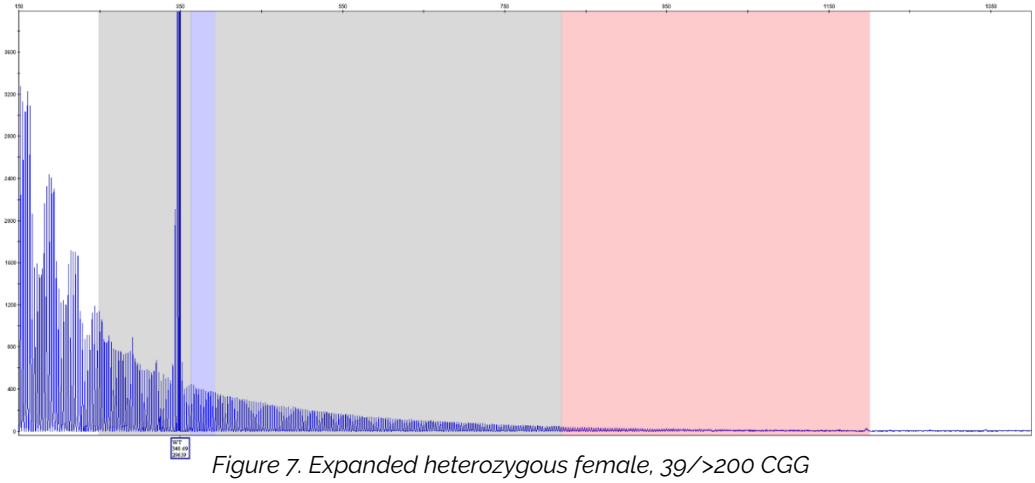


Figure 7. Expanded heterozygous female, 39/>200 CGG

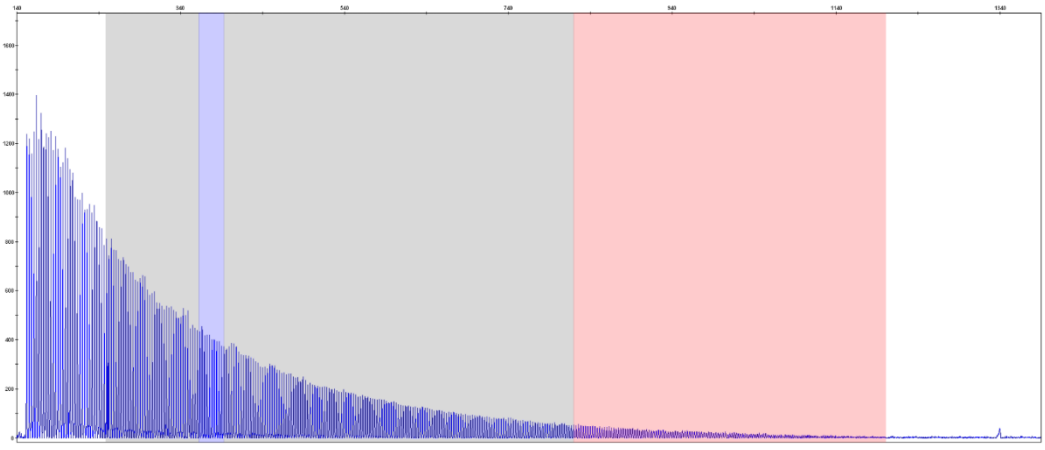


Figure 8. Expanded male, >200 CGG

Primers of the Adellgene® Fragile X are specific for CGG repeats and do not hybridize to AGG sequences commonly found within the *FMR1* gene. Accordingly, the signal valleys that appear on the electropherogram correspond to the presence of interspersed AGG regions.

The AGG interruptions are thought to confer DNA stability and to diminish the risk of expansion in the offspring [8]. Figure 9 shows a healthy female sample yielding the pattern composed by CGG and AGG triplets as indicated.

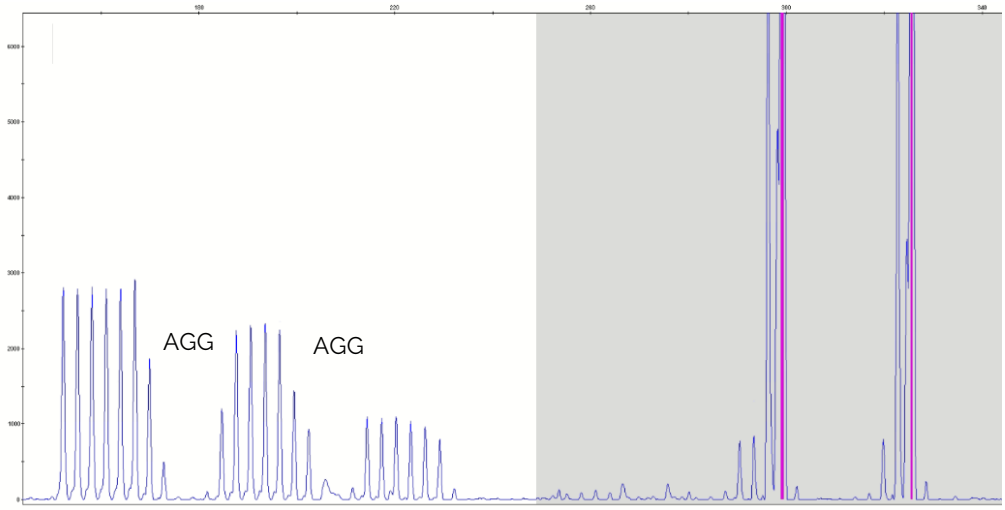


Figure 9. Healthy female sample showing the characteristic pattern composed by CGG and AGG triplets

In case of obtaining a saturated peak in the electropherogram, a new injection could be done diluting the PCR products (1:100, 1:200), to set the peak size more accurately.

11 – Quality control

Given the quantitative nature of this test and with the aim of ensuring correct detection of the fluorescent signal, the fluorophores FAM and ROX must be calibrated in the genetic analyser.

To be able to correctly measure the size of the alleles, the molecular weight marker must be added to all wells. All molecular weight marker peaks must be detected for all samples (including the negative control).

In each round of analysis, a no template control should be added to ensure the assay is contaminant free.

12 – Specific operation data

➔ Analytical specificity

This kit's primers are specific to the human Fragile X Mental Retardation gene (*FMR1*) (NC_000023.10) and comprise the CGG repeat region within the 5'UTR of the gene (NT_011681. 16). The specific amplification of this region was verified by DNA sequencing and testing samples from healthy individuals and well-characterised patients. No cases of cross-reactivity with another gene from genomic DNA have been reported.

➔ Analytical sensitivity

With the aim of determining the most appropriate amount of DNA for this test, some tests were carried out analysing five different samples (healthy, premutation and expanded females, and premutation and expanded males), between 10 ng and 200 ng. Assignment of the peak size obtained was independent to the amount of DNA used. The recommended working range is between 20 and 80 ng of DNA.

➔ Diagnostic specificity.

Adellgene® Fragile X is a specific test to detect the number of CCG repeats in the 5'UTR of the *FMR1* gene. The kit allows the quantification of healthy and premutation alleles consisting of a number of repeats up to 200. It also allows the visualisation of expanded alleles (>200 CGG repeats).

Mutations (point mutations, insertions, deletions) at hybridisation points of the amplification primers are possible and may lead to a lack of definition of an allele. Other technologies may be required to resolve the alleles in these cases.

Diagnostic specificity was assessed in one external and one internal study, analysing a total of 34 samples with the following results:

Sample type	No. of analysed samples
2 normal alleles (up to 45 or 55 CGG repeats)	7
1 premutation allele (45 or 55-200 CGG repeats)	18
1 expanded allele (>200 CGG repeats)	9
Total number of samples analysed	**34**

All results coincided with the prior information available on these samples, which were previously analysed using the routine method of the source laboratory.

➔ Diagnostic sensitivity.

The Adellgene® Fragile X kit can assign a number of CGG triplet repeats up to 200. This allows the alleles present in the sample of an individual to be classified as healthy (up to 45 or 55 CCG), premutation (45 or 55 to 200 CGG) or expanded (>200 CGG).

➔ Accuracy

Interferences

Existing literature has described a number of substances that may be present in peripheral blood and which may potentially interfere in the PCR, inhibiting polymerase's activity. Thus, before proceeding with the test, we recommend assessing the DNA's purity. The majority of standard extraction methods allow these substances to be eliminated, for which reason we also recommend validating the extraction method used before proceeding with the test.

Trueness

The precision in the number of calculated repeats was determined by comparing the sizes obtained using the Sanger sequencing technique with this kit. Thus, an acceptable variability of ±1 repeats was established for healthy alleles and ±3 for alleles that contain 50-200 repeats.

13 – Procedure limitations

- This kit can quantify all alleles up to 200 CGG repeats.
- Mutations (point mutations, insertions, deletions) in complementary regions to the primers sequence are possible and may lead to a lack of definition of an allele. Other technologies may be required to resolve these alleles.

- All the recommendations mentioned in this document should be carefully followed. Any performances that do not meet such indications, can lead to poor results.
- Do not use the kit if there are any suspicions of possible loss of reactivity, contamination, external box deterioration or any other incidence that might affect the kits performance.
- All Adellgene® reagent manipulations must be done according to the general good practices of laboratory, being adapted to local regulations.
- Do not mix components from other kits or lot numbers.
- Do not use the kit after its expiry date. Dispose expired reagents according to applicable regulations.
- The thermal cycler must be calibrated according to the manufacturer´s recommendations and should be used accordingly to the manufacturer's instructions.
- Data and result interpretation should be revised by qualified personnel.
- This product is an auxiliary tool for the diagnosis of patients with suspected disorders related to *FMR1* gene. Use these results in conjunction with clinical data and results of other tests performed on the patient

14 – Troubleshooting guide

➔ The negative control shows a positive result

- Contamination of the Primer Mix of Polymerase Mix or the negative control's water
    - Repeat the experiment with new aliquots of the Primer Mix/Polymerase Mix/water.
    - Handle the components of the kit provided in accordance with commonly accepted practices to prevent contamination.
    - Check how the kit is stored and the handling conditions.
    - Dispose of contaminated reagents.
- Contamination in the pre-PCR area
    - Check the necessary precautions have been followed in the pre-PCR area.
    - Check possible contamination issues in other PCR techniques.
    - Check the suitability of the fungible products used (tubes, pipette tips).
- Pipetting error
    - Check the added sample corresponds with the assigned samples on the work sheet.

➔ Incorrect band size or incorrect number of bands

- Incorrect use of the kit
    - Check the correct kit has been used.
- Use of an incorrect thermal cycling program
    - Check the settings of the thermal cycler.

- Incorrect size standard used in the capillary electrophoresis or wrong detection of the peaks.
    - Check the size standard used is correct.

- Contamination of the PCR
    - Check the negative control of the run.
    - Carry out decontamination protocols and repeat the PCR to identify the source of contamination.

- Incorrect settings used in the genetic analyser
    - Check the module of the genetic analyser.

➔ Weak signal in the electropherograms

- Low quality or concentration of PCR product
    - Adjust the concentration of DNA to the recommended values for this test.
    - Avoid using whole blood that contains heparin. If necessary, repeat DNA extraction and the PCR.

- Insufficient amount of PCR product injected in the DNA analyser
    - Adjust the amount of PCR product

- Insufficient amount of Primer Mix and/or Polymerase Mix added, or deficient mixing of the PCR components.
    - Repeat the PCR, ensuring that all components have been mixed correctly and the right amount added.

- The Taq polymerase has lost activity
    - Confirm the activity of the Polymerase Mix.
    - Repeat with a new vial of Polymerase Mix.

- Degradation of the kit
    - Confirm the kit has been stored in line with the manufacturer's instructions.
    - Do not freeze/unfreeze the reagents more than two times.
    - Prepare aliquots of the reagents if necessary.
    - Repeat the analysis with a fresh batch of reagents.

➔ High intensity of fluorescence

- Excess of PCR product
    - Dilute the PCR product.

- Incorrect settings used in the genetic analyser
    - See above.

- Pipetting error
    - Check the volume added to each well is correct.

➔ High background signal (noisy baseline)

- Contamination of the PCR

- See above.

- Low quality of PCR product
  - See above.

15 – References

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16 – Notice to purchaser

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17 – Changes control

Version	Description of the modification
Rev. 02	Correction of writing and translation errors. New sections: *Accuracy*, *Information for safety*, *Changes control* and *Explanation of symbols used on the labels*. Information related to the intended user and intended patient. New document format.

18 – Explanation of symbols used on the labels

IVD	*In vitro* diagnostic medical device		Expiration date
REF	Catalogue number	Σ	Contents sufficient for <n> tests
LOT	Lot number		Manufacturer
	Temperature limit		Keep away from sunlight
CONTROL +	Positive control		Consult electronic Instructions For Use document
CE	This product fulfils the requirements of Directive 98/79/EC on in vitro diagnostic medical device		