

Elucigene[®] CF4v2 Instructions for Use

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ELUCIGENE kits are developed and manufactured by Gen-Probe Life Sciences Ltd. within quality systems accredited to ISO9001:2008 and ISO13485:2003.

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Elucigene CF4v2

Catalogue Code: CF4HTB1 – 25 tests

Intended Use

For the simultaneous *in vitro* qualitative detection of human Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene mutations G542X, F508del, 621+1G>T and G551D in human whole blood. The test can distinguish between individuals who are heterozygous and homozygous for the all the above mutations.

Principles of the procedure

The method employed by the Elucigene CF4v2 kit uses fluorescent ARMS™ allele specific amplification technology, which detects point mutations or small deletions in deoxyribonucleic acid (DNA). The principle of ARMS™ is that oligonucleotides with a 3' mismatched residue will not function as Polymerase Chain Reaction (PCR) primers under specified conditions. Selection of appropriate oligonucleotides allows specific mutant or normal DNA sequences to be amplified and detected.

Warnings and Precautions

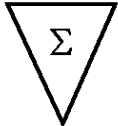
1. For professional *in vitro* diagnostic use only.
2. The normal DNA Control provided in this kit has been independently tested and found to be negative for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) 1 and 2.
3. Care should be taken when handling material of human origin. All samples should be considered potentially infectious. No test method can offer complete assurance that HBV, HCV, HIV or other infectious agents are absent.
4. Licences for the *in vitro* diagnostic analysis of gene mutations detected by these reagents may be required and are the responsibility of the reagent purchaser.
5. Handling of samples and test components, their use, storage and disposal should be in accordance with the procedures defined by the appropriate national biohazard safety guideline or regulation.
6. Store primer mix below -20°C and extraction buffer at 4°C. Discard 3 months after opening unless sub-aliquoted
7. In line with current good laboratory practice, laboratories should process their own internal QC samples of known genotype in each assay, so that the validity of the procedure can be assessed.

Symbols used on labels

The symbols used on all labels and packaging conform to the harmonised standard EN980



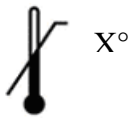
Manufacturer



Number of tests



See Instructions for Use



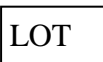
Store below temperature shown



Use before date shown



Catalogue code



Lot or batch number

Materials Provided

1 x 250µL vial of reaction mix containing primers to detect the following 4 CFTR mutations: G542X, F508del, 621+1G>T and G551D. The Reaction Mix also contains wild type primers for the detection of each normal allele, DNA polymerase and deoxynucleotide triphosphates in buffer.

1 x 50µL vial DNA Control, normal for the mutations detected by Elucigene CF4v2.

Materials required but not provided

General

Laboratory consumables – gloves; screw-capped eppendorfs; microfuge tubes; 96well 0.2mL PCR plates or 0.2ml PCR tubes; pipette tips.

Laboratory equipment – precision pipettes (2 sets: 1 for pre-amplification and 1 for post-amplification handling: - preferably positive displacement pipettes); protective clothing; vortex mixer; microfuge; 96 well microtitre plate centrifuge.

DNA Extraction

DNA preparation – QIAamp® DNA Mini Kit (Qiagen Ltd, Cat No 51304/6) or equivalent kit

PCR Amplification

Thermal cycler to accommodate 96 well microtitre plates or 0.2mL vials with a temperature accuracy of +/-1°C between 33°C and 100°C and static temperature uniformity of +/-1°C.

Capillary Electrophoresis

Capillary Electrophoresis - GS400HD ROX size standard, Multi-capillary DS-30 (dye set D) matrix standard, POP-6/POP-7, 10x Genetic Analyzer Buffer and Hi-Di Formamide. ABI 3100/3130 Genetic Analyzer (with fragment analysis software), 36cm capillary array, 96 well optical plates, 96 well septas, 96 well cassettes.

Sample Collection and Storage

Whole blood (EDTA) or dried bloodstains should be used. Sample collection devices have on occasion been reported to be detrimental to the integrity of certain analytes and could interfere with some method technologies. It is recommended that each user ensure that the chosen device is used according to the manufacturer's instructions and both sample collection devices and DNA preparation methods are compatible with this test.

DNA Extraction of whole blood (EDTA) and bloodspots

The QIAamp® DNA Mini Kit is recommended. For less concentrated DNA samples a higher volume of water can be used for eluting. For more concentrated DNA samples a lower volume of water can be used for eluting.

DNA concentration

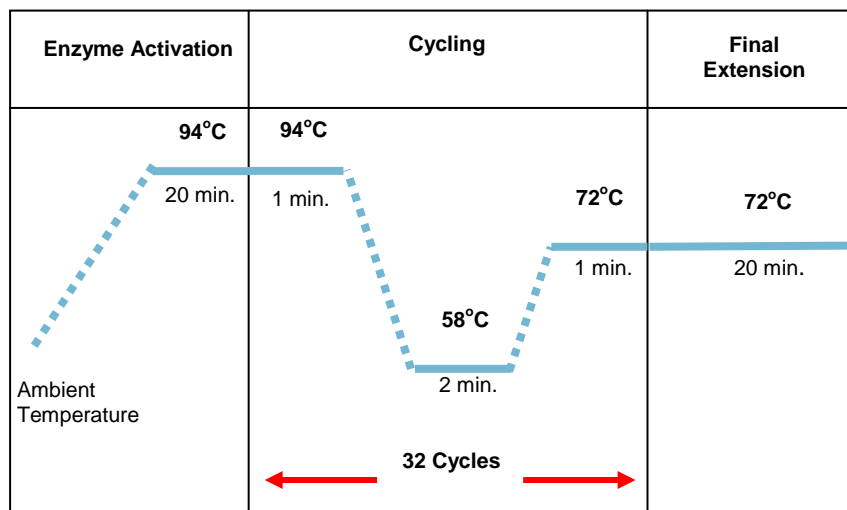
It is recommended that alternative DNA extraction methods and sample types are thoroughly evaluated with the Elucigene CF4v2 test prior to the results being used for diagnostic use. Under optimal PCR conditions and using the recommended sample injection settings* stated in the capillary column run modules, results are consistently obtained at DNA amounts between 0.5ng and 5ng.

***Note:** Sample injection settings can be modified to suit the amount of amplicon produced during the PCR reaction which can vary due to amount of input genomic DNA added. Less amplicon can be applied to the column for analysis by reducing either time or voltage of injection. Conversely more amplicon can be applied to the column for analysis by increasing either time or voltage of injection. Previously amplified samples can be re-injected multiple times for re-analysis.

Amplification Procedure

Note: To minimise the risk of contamination, steps 2 - 5 must be carried out in an area free from DNA. Steps should also be taken to avoid contamination with PCR product. A negative control must be included in each PCR run.

1. Program the thermal cycler for a single step cycle to activate the DNA polymerase at 94°C for 20 minutes linked to an amplification cycling program of 1 minute at 94°C (denaturation), 2 minutes at 58°C (annealing) and 1 minute at 72°C (extension) for 32 cycles. This should be linked to a 20 minutes time-delay file at 72°C (extension) on the final cycle.



2. Thaw and centrifuge the reaction mix for 10 seconds at 12,000 x g, mix gently by vortexing and centrifuge the vial again for 10 seconds.
3. Pipette 10µL of reaction mix into the bottom of the appropriate well of a 96 well plate (or 0.2mL PCR tubes).
4. Using separate pipette tips add 2.5µL of test DNA sample to each of the wells (or tubes) and cap. Do not add DNA to the well for the negative control, substitute with 2.5µL of sterile deionised water.
5. Centrifuge the PCR plate (or tubes) for 10 seconds at 2,000 x g.
6. Place the plate (or 0.2mL PCR tubes) firmly in the thermal cycler block. Initiate the 94°C single step cycle followed by the amplification cycling program.
7. On completion of the amplification cycling program, the samples may be stored at room temperature overnight or at 2-8°C for up to 7 days before analysis by capillary electrophoresis.

Capillary Electrophoresis

It is recommended that each user ensure that the chosen equipment is used according to the manufacturer's instructions and is compatible with this test. In this context the key parameters are the polymer and the capillary array. Optimal results can be obtained using the following capillary electrophoresis conditions:

1. Combine 6.0µL of GS400HD ROX size standard with 340µL Hi-Di Formamide and mix thoroughly (sufficient mix for 16 wells). Dispense 20µL of the mix into each well of a 96 well optical plate.
2. Add 2µL of PCR product to the size standard mix (from step 1) already dispensed into the plate.

3. Denature the PCR product dispensed into the optical plate on a thermal cycler using the following parameters: 94°C for 3 minutes linked to 4°C for 30 seconds.
4. Centrifuge the plate at 1,000g for 10 seconds to remove any bubbles in the wells and load onto the Genetic Analyzer.

ABI 3100 Instruments:

Create a sample sheet using the 3100 data collection software with the following settings:

- Sample Name: This must be a sample specific name or number.
- Size Standard colour: Ensure the red coloured box is marked with a diamond in order that GS400HD Rox may be identified as the size standard.
- Colour Info: Copy and paste sample name column data into this column.
- Colour comment: Denote the CF4v2 mix has been used to amplify the sample
- Dye Set: D
- Run Module: 36cm capillary run module see below.*

***Note:** Required 'run time' will vary dependent on the ambient temperature of the location in which the Genetic Analyzer has been installed. For more information on creating run modules please refer to your instrument user manual.

ABI 3130 Instruments:

Create a sample sheet using the 3130 data collection software with the following settings:

- Sample Name: This must be a sample specific name or number.
- Run Owner: Select the default owner for lab.
- Run Protocol: CFHT4 (contains CF4v2 3130 run module – see below) *

***Note:** It is necessary to create a run module detailing the instrument settings and subsequently assign this to a Run protocol in which Dye set D has been selected. For more information on creating run modules please refer to your instrument user manual.

3100 Run Module

For POP6 Polymer

36cm Capillary Module: 36CFHT4

#	Parameter Name	Value	Range
1	Run Temperature	60	int 18...65 Deg.C
2	Cap Fill Volume	184	int 1...200 steps
3	Current Tolerance	100	int 1...100 uAmps
4	Run Current	100	int 1...200 uAmps
5	Voltage Tolerance	0.6	float 0.25...2.0 kVolts
6	Pre Run Voltage	15	float 0...15 kVolts
7	Pre Run Time	180	int 1...1000 sec.
8	Injection Voltage	3	float 1...15 kVolts
9	Injection Time	3	int 1...600 sec.
10	Run Voltage	15	float 0...15 kVolts
11	Number of Steps	10	int 1...100 nk
12	Voltage Step Interval	60	int 1...60 sec.
13	Data Delay Time	1	int 1...3600 sec.
14	Run Time	950	int 300...14000 sec.

3130 Run Module

For POP7 Polymer




36cm Capillary Module: CFHT4

#	Parameter Name	Value	Range
1	Oven Temperature	60	int 18...65 Deg.C
2	Poly_Fill_Vol.	6500	6500...38000 steps
3	Current Stability	5.0	int 0...2000 uAmps
4	PreRun_Voltage	15.0	0...15 kvolts
5	Pre_Run_Time	180	1...1000 sec.
6	Injection_Voltage	3.0	1...15 kvolts
7	Injection_Time	3.0	1...600 sec.
8	Voltage_Number_Of_Steps	20	1...100 nk
9	Voltage_Step_Interval	15	1...60 sec.
10	Data_Delay_Time	60	1...3600sec.
11	Run_Voltage	15.0	0...15 kvolts
12	Run_Time	700	300...14000 sec.

Interpretation of results

During 3100/3130 data collection, PCR fragments will be observed as either Blue (mutant) or Green (wildtype) peaks on the Raw Data electropherogram. An individual has two copies of the CFTR gene. Where these copies have the same sequence for any given site, an individual is described as being homozygous for this site. Where the copies differ in sequence at a given site, an individual is described as being heterozygous for this site. Peaks observed for any specific allele for the two copies of the CFTR gene are displayed below.

CF4v2 Peak Morphology

	Homozygote Wild Type	Heterozygote	Homozygote Mutant
Peaks observed	Single Green peak	Green & Blue Peak	Single Blue peak
Peak Morphology			

The figure shown emphasises equivalent peak heights, but note that peak positions will be of almost equal base pair size.

Markers detected

Once data collection has finished, CF4v2 PCR fragments should be sized against the GS400HD ROX size standard using fragment analysis software. Table 3 summarises the markers detected by CF4v2 mix. Markers are listed according to the potential size range of PCR product observed. It is recommended that these size ranges be used when assigning marker detection limits. For all markers at least one peak should be present.

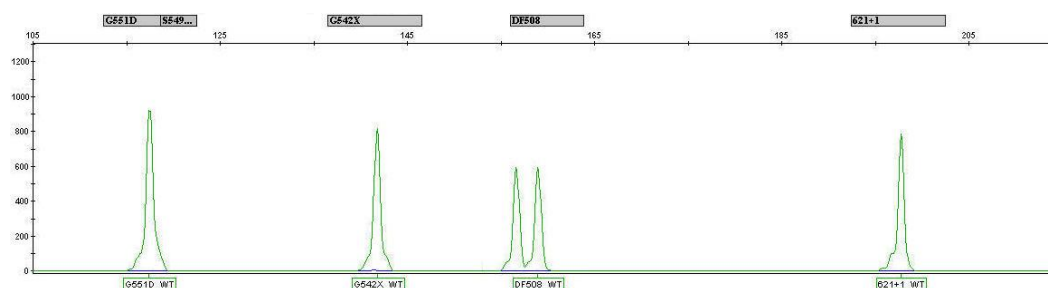
Table 3: Markers Detected

Marker #	Marker	Product bp Size Range
01	G551D	112.5 - 122.5
02	G542X	136.5 - 146.5
03	F508del	156.0 - 163.8
04	621+1G>T	192.4 - 202.4

Note: Size ranges for each marker may vary due to the polymer and size standard used.

I507del

Due to the location of the I507del deletion it is detected by the F508 WT primers. Presence of this allele appears as a green peak which is 3bp smaller in size than the F508 WT peak (see below).



This figure shows the I507del peak to the left of the F508del peak.

Note: One blue and one green dye related artefact migrating between G542X and F508del peaks may be observed in weak samples. These artefacts will not be sized and can be excluded from analysis.

Cross Reactivity

The following rare mutations have been evaluated for cross reactivity and were not detected by the Elucigene CF29 kit (which uses the same primer sequences): 621+2T>C and I506V. In addition the following polymorphisms were not detected by the test: 1655T/G (F508C), 1651A/G.

The following mutations, which have not been checked due to unavailability of relevant samples, may interfere with test function: 621+2T>G, R553G, R553Q, I506T and I506S.

1. **The G551D mutant primer will cross react with S549RT>G mutant sequence and result in a diagnostic peak 2bp larger than the expected size of the G551D mutant peak. This peak will be labelled as S549RT>G_M. There will also be a reduction in the height of the G551D wild-type peak.**

Limitations of the Procedure

1. The results obtained from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. Gen-Probe Life Sciences Ltd is not responsible for any clinical decisions that are taken.
2. The absence of the mutations detected by this kit is no guarantee that other mutations in the CFTR gene are not present. Many other mutations are possible and are not detected by this kit.
3. Mutations vary in frequency between different populations. Population mutation frequency data is available from The Cystic Fibrosis Genetic Analysis Consortium.
4. The user of this kit should emphasise these points when reporting results to the diagnosing clinician/genetic counsellor.