ChromoQuant® QF PCR kit v.3 Instructions For Use – edition E13

Intended use

ChromoQuant® QF-PCR kit (P/N 311.003 and 412.002) is a rapid prenatal test for detection of the three most common autosomal trisomies; Trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome) and Trisomy 13 (Patau syndrome). *ChromoQuant*® (P/N 311.003) will also detect aneuploidies in chromosomes X and Y such as Klinefelter syndrome and Turner syndrome.

The *ChromoQuant*® kits are intended for professional *In Vitro* diagnostic use only.

Extra marker kits (P/N 313.003, 318.003, 321.003 and 330.002) are available for extended chromosome testing. The extra marker kits are complements to 311.003 and 412.002 and hence cannot be used as stand alone products.

ChromoQuant® has been developed to be used with DNA extracted from Amniotic Fluid or Chorionic Villus samples. DNA from whole blood or saliva can also be used with the ChromoQuant kit.

ChromoQuant products

ChromoQuant® is a registered trademark of CyberGene AB, Sweden.
ChromoQuant® kits are developed and manufactured by CyberGene AB, within quality systems accredited to ISO 13485:2003 and ISO 9001:2008.

Customer information available at www.cybergene.se/chromoquantQFPCR.htm

Customer support

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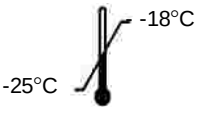
Expiry date
See package

Lot no./ See package

Number of tests /
See package



Recommended storage temperature


Protect from sunlight


Production date


Produced by

The ChromoQuantQF-PCR kit is always shipped at ambient temperature

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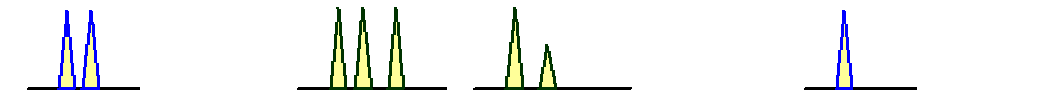
Principles behind the QF-PCR method

The technology employed by *ChromoQuant*® kits is the QF-PCR method (Quantitative Fluorescent Polymerase Chain Reaction). Highly specific STR (short tandem repeat) regions within chromosomes 13, 18, 21 and X/Y will be targeted by genetic markers. The PCR-products will contain fluorescent labels. Hence, during electrophoresis, these products will generate bands of different colours, within well defined size ranges (base pairs). These bands will have a pattern reflecting the genetic status of the sample.
A normal informative diploid sample will generate two bands of the same intensity (1:1), reflecting two alleles of each marker on the chromosomes of interest. A homozygote sample, where both alleles are of the same size, will generate a single, non-informative band.
Three bands of the same intensity (1:1:1 ratio) or a 2:1 ratio will reflect the presence of an extra chromosome.



Normal heterozygote 1:1 Trisomy 1:1:1 Trisomy 2:1 Homozygote non-informative

QF-PCR is a quantitative method. In this case this means that the areas and/or the heights of the peaks are of comparable sizes, relating to the number of chromosomes of interest present in the sample. Therefore, the ratios of the areas and/or the heights are studied.
Normal peak ratios within an STR is 0,8-1,4. Trisomy peak ratio is <0,65 or >1,8, i.e. the area or height of the shorter length allele divided by the area or the height of the longer length allele. Allele ratios that fall between the normal and abnormal ranges are classed as inconclusive and must be re-analysed. If both abnormal and normal allele patterns are obtained for a single chromosome, it is unacceptable to interpret the final result as normal or abnormal. Follow-up studies must be carried out.

For detailed information about interpretation of results please refer to User's Guide for ChromoQuant. The User's Guide can be downloaded from ChromoQuant web site or ordered directly from CyberGene AB. Product # 901.115-00.

Limitations of the method and performance characteristics

In the 311 kits the marker information from chromosomes 13 are 5x, 18 are 6x and chromosome 21are 6x redundant. In the 412 kits all markers are 4x redundant and the sex chromosomes are not analysed.
An analytical result where two of the markers for a particular chromosome are informative should be considered as successful and can be used as a diagnostic indication.
The kits are not intended for evaluating or determining if a translocation has occurred, involving chromosomes 13, 18, 21, X or Y. The kit must not be used in order to assess mosaic genetic aberrations (e.g. lyonization). Mosaicism may not be detected. *ChromoQuant*® will give a strong indication for Turner X0 syndrome. Additional investigation might be necessary. Discrepancies between *ChromoQuant*® and karyotyping results may depend upon maternal contamination or confined placental mosaicism.

Precautions

- For use with fluorescent sequencers supporting filter set D (6-FAM, HEX, NED and ROX) for Applied Biosystems ABI PRISM®Genetic Analyzers, and filter set 2 for Amersham Biosciences MegaBACE™ systems.
- Make sure that your sequencer will support the dye labels 6-FAM, HEX and NED.
- This kit is recommended for use with DNA purified from amniotic fluid, chorionic villus, mouth swabs, saliva or whole blood.
- The function of the kit cannot be guaranteed unless using one of the validated Taq Polymerases. Taq polymerase is NOT included in the kit.
- This QF-PCR protocol has been optimised after using the InstaGene Matrix (BioRad #732-6030) purification method. Other methods may be used. A very pure DNA must be diluted to a lower

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concentration than the recommended value. We strongly recommend internal validation and optimization before taking the ChromoQuant kit into clinical use.

- For optimal PCR result – follow instructions given for available PCR instrument.
- Blood stained patient samples should not be analysed with this kit e.g. when using DNA from amniotic fluid! If a blood stained sample is used, then the DNA of the mother must be analysed and used as a control.
- In order to avoid cross contamination during PCR setup - make sure that you have changed to a new pipette tip for each well to be filled with DNA.
- Salt might interfere with the fragment separation during gel electrophoresis. For MegaBACE users we strongly recommend desalting after PCR.
- Quality of Formamide or water added to the DNA sample before electro kinetic injection will affect the sensitivity. Be sure to use the highest quality.

Kit Composition

ChromoQuant kits contain tubes with primer master mix solutions with genetic markers for specific chromosomes according to the table below.

For the 311-kit the tube with white cap (311.322) must be used to determine chromosome 13/18. The tube with blue cap (311.323) must be used to determine chromosome 21. In order to analyse the sex chromosomes using the 311-kit, both tubes must be used. The content of the Extra Marker tubes will analyze 10 samples.

The solutions do not contain any Taq polymerase. This must be added prior to use according to instructions under "Procedure for pre-dispensing and PCR".

Part of Kit #	*Solution #*	*No. of tests*	*Cap colour*	*Analysed chromosomes*
311.003-52/-24	*311.322-52/-24*	*52/24*	*White*	*Chromosomes 13, 18, X, Y*
311.003-52/-24	*311.323-52/-24*	*52/24*	*Blue*	*Chromosomes 21, X, Y*
412.002-24	*412.324-24*	*24*	*Clear*	*Chromosomes 13, 18, 21*
311.003-52 *313.003-10*	*313.003-10*	*10*	*Red*	*Extra markers Chromosome 13*
311.003-52 *318.003-10*	*318.003-10*	*10*	*Violet*	*Extra markers Chromosome 18*
311.003-52 *321.003-10*	*321.003-10*	*10*	*Yellow*	*Extra markers Chromosome 21*
311.003-52 *330.002-10*	*330.002-10*	*10*	*Green*	*Extra markers Chromosomes X, Y*

The Extra markers are available separate but must not be used as stand alone products.

Storage instructions and shelf life after first opening

- Store unused primer master mix solution in the dark at -18°C to -25°C.
- It is recommended to always store unused material in the original box.
- Shelf Life: See expiry date

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Additional special reagents required to be supplied by the user

- Taq polymerase (see list of validated enzymes below)
- Gel-electroforesis fluorescent size standard, e.g. ABI GeneScan®-500 [ROX]™ product #401734 or GE Healthcare ET-550R product #25-6550-01.This is added to the DNA sample after PCR but before gel electrophoresis.
- Other reagents and equipment related to the Electrophoresis.
- Other reagents for purification of genomic DNA or desalting of PCR products.

Validated Taq polymerases

- Hot Start: HotStar Taq polymerase, *Qiagen #203203, 250U* [5 U μL^{-1}]
- Hot Start: True Start Taq polymerase, *Fermentas* #EP0612, 500 U [5 U μL^{-1}]
- Go Taq polymerase, *Promega* (not Hot Start!) #M8305, 500 U [5 U μL^{-1}] ***Work on Ice!***
  Note: *Go Taq polymerase not recommended for the 412-kit.*

Procedure for pre-dispensing and PCR

DNA purification and preparation

Reagents for purifying DNA from human tissue are NOT included in the kit. It is recommended to use commercial DNA preparation technologies providing a pure genomic DNA. A very pure DNA might be diluted to a lower concentration than the recommended value below. For information about purification method please refer to the User´s Guide. Internal optimization might be necessary depending upon the purification method used.

1. Thaw the ChromoQuant pre-mix mastermix tubes to room temperature
2. Add Taq polymerase (see above) according to the schedule below:

Solution	Cap colour	Taq Polymerase (µL) 5U/µL
311.322-52	white	22,0
311.323-52	blue	22,0
311.322-24	white	10,4
311.323-24	blue	10,4
412.324-24	clear	10,4

Solution	Cap colour	Taq Polymerase (µL) 5U/µL
313.003-10	red	4,4
318.003-10	violet	4,4
330.002-10	green	4,4

3. Vortex and make sure that the reagents are properly mixed with the Taq polymerase. If this is not done correctly the mix might not freeze upon storing.
4. Spin down
5. Aliquot 15 µL of master mix from step 2 to sterile PCR tubes of different colour (0,2 ml tubes) . Make sure to separate the various solutions when storing. Use separate pipett tips for all solutions.
6. Store the ready-to-use PCR tubes in a freezer between -18°C and -25°C

For optimal performance. Avoid too many freeze/thaw cycles as this will break down the dye material and cause weak signals at analysis. For optimal performance we recommend to aliquot the solutions immediately and to store the material in a freezer before use.

Product	Pre-dispensed volume (µL)	$DNA_{(aq)}$ (µL)	Final volume (µL)
All PCR tubes	15	10	25

Product	Master mix per test (µL)	Taq polymerase (5U/µL) per test (µL)	Diluted DNA (µL)	Final volume (µL)	This schedule is used if the solutions have not been dispensed into PCR tubes in advance by the user. However, we strongly recommend pre-dispensation.
All tests	14,6	0,4	10	25	

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PCR set up

1. Take out as many tubes of each type as you wish to use and thaw on ice
2. Add 10 µl of diluted DNA (1,5-15 ng/µL). Mix by pipette pumping 5X. *Final volume 25* µL
3. (Suggestion: From InstaGene matrix (BioRad) purified Amniotic Fluid take 5 µL+5 µL sterile water, from CVS sample take 2,5 µL+ 7,5 µL sterile water)
4. Spin down in a micro centrifuge to make sure all reagents are collected in the bottom of the PCR tube.
5. Immediately run PCR amplification.

Choose step one according to what Taq polymerase is used.

Step	No. of cycles	Temp.	Time
1. Hot Start	***1***	***95 °C***	***15 min***
1. GoTaq	***1***	***94 °C***	***3 min***
2	Repeat 26	94°C	30 sek
3	"	57°C	1 min
4	"	71°C	2 min
5	1	71°C	5 min
6	1	60°C	1 h
7	HOLD	4 °C	∞

Gel electrophoresis of amplified DNA fragments

1. Run gel electrophoresis according to instrument manual or best-practice in the lab for best resolution of fragments between 93 – 500 bp
   See the user´s guide for more detailed information about set up recommendations.
2. Desalting the PCR product before electrophoresis is strongly recommended for MegaBACE users.
3. See product insert or the User´s Giude for additional Information about capillary Instrument set up.

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Kit #311.003-52 / 311.003-24

Solution 1 (#311.322) – chromosomes 13 and 18 and X/Y – white cap

Identity	Location	Chromosome	STR	Size bp	Color
AMEL	Xp22.31-Xp22.1 Yp11.2	X/Y (AMEL)	-	x: 103-108 y: 109-114	Green
D13S797	13q33.2	13	Tetra	175-250	Blue
D18S391	18p11.31	18	Tetra	135-185	Green
D18S976	18p11.31	18	Tetra	166-201	Yellow
D18S819	18q11.2	18	Tetra	230-275	Yellow
XHPRT	Xq26.1	X	Tetra	260-304	Blue
D13S742	13q12.13	13	Tetra	230-326	Green
DXYS218	Xp22.32/Yp11.3	X/Y	Tetra	310-350	Blue
D18S390	18q22.3-18q23	18	Tetra	315-360	Yellow
D18S386	18q22.1	18	Tetra	330-405	Green
D13S634	13q21.33	13	Tetra	380-445	Blue
D13S628	13q31.1	13	Tetra	420-475	Yellow
D13S305	13q13.3	13	Tetra	425-470	Green
D18S535	18q12.3	18	Tetra	450-505	Blue

Solution 2 (# 311.323) – chromosome 21 and X/Y – blue cap

Identity	Location	Chromosome	STR	Size bp	Color
DXS6854	Xq26.1	X	Tetra	91-119	Blue
DXS6803	Xq21.31	X	Tetra	101-139	Green
D21S1409	21q21.2	21	Tetra	189-219	Yellow
SRY	Yp11.31	Y (SRY)	-	202-207	Blue
X22	Xq28Yq	X/Y	Penta	190-250	Green
D21S11	21q21.1	21	Tetra	224-270	Yellow
D21S1246	21q22.2	21	Tetra	282-336	Green
D21S1411	21q22.3	21	Tetra	283-344	Yellow
D21S1444	21q22.13	21	Tetra	300-345	Blue
D21S1435	21q21.1	21	Tetra	350-410	Green

Kit #313.003

Extra markers Chromosome 13 (#313.003) – red cap

Identity	Location	Chromosome	STR	Size bp	Colour
D13S325	13q14.11	13	Tetra	193-231	Blue
D13S762	13q31.3	13	Tetra	270-326	Green
D13S305	13q13.3	13	Tetra	425-470	Green

Kit #318.003

Extra markers Chromosome 18 (# 318.003) – violet cap

Identity	Location	Chromosome	STR	Size bp	Colour
D18S878	18q22.1	18	Tetra	153-192	Green
D18S1002	18p11.1	18	Tetra	280-370	Blue

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Kit #321.003

Extra markers Chromosome 21(# 321.003) – yellow cap

Identity	Location	Chromosome	STR	Size bp	Colour
D21S1409	21q21.2	21	Tetra	189-219	Green
D21S1411	21q22.3	21	Tetra	283-344	Blue
D21S1280	21q22.11	21	Tetra	316-386	Green

Kit #330.002

Extra markers Chromosomes X and Y (#330.002) - green cap

Identity	Location	Chromosome	STR	Size bp	Colour
DXS6803	Xq21.31	X	Tetra	101-139	Green
DXS8377	Xq28	X	Tri	198-280	Green
DXS6809	Xq21.33	X	Tetra	230-285	Blue
G10_STS47	Yq11.222	Y	--	338-343	Green

Kit #412.002-24

Solution 1 (#412.324) Chromosomes 13, 18 and 21 – single tube test – clear cap

Identity	Location	Chromo-some	STR	Size bp	Colour
D18S391	18p11.31	18	tetra	135-185	Green
D21S1435	21q21.3	21	tetra	150-210	Yellow
D18S976	18p11.31	18	tetra	166-201	Blue
D21S11	21q21.1	21	tetra	224-270	Yellow
D21S1444	21q22.13	21	tetra	226-267	Blue
D13S742	13q12.13	13	tetra	230-326	Green
D21S1246	21q22.2	21	tetra	282-336	Blue
D18S386	18q22.1	18	tetra	330-405	Green
D13S634	13q21.33	13	tetra	380-445	Blue
D13S628	13q31.1	13	tetra	420-475	Yellow
D13S305	13q13.3	13	tetra	425-470	Green
D18S535	18q12.3	18	tetra	450-505	Blue

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Historic Changes - Track Record

The *ChromoQuant*® kit is continuously improved based upon comments and suggestions from our customers. If you have any suggestions – please contact our support at chromoquant@cybergene.se

Changes in Instructions for Use version 12 compared to version 11

PCR protocol: 1 min, 94°C, 3 min added in step 1 for GoTaq polymerase
D13S325 changed size from 195-231 to 193-231 due to verified alleles outside range.

Changes in version 311.002.2 compared to 311.002.1

The following changes have been introduced in the new version of ChromoQuant:

4 new markers are introduced in the analysis of chromosome 13, 18, X/Y (orange tubes);

D13S256	13q14.1-13q22	13	Tetra	125-175	Blue
D18S819	18q11.2	18	Tetra	230-275	Yellow
DXYS218	Xp22.32/Yp11.3	X/Y	Tetra	310-350	Blue
D18S390	18q22.3-18q23	18	Tetra	315-360	Yellow

Changed fluorescent dye in one marker (from 6-FAM to NED);

D18S976	18p11.31	18	Tetra	171-201	Yellow

Also, new PCR tubes are used. The reason for this is that these tubes fit better into the holder and accordingly, are easier to remove.

Changes in version 311.003 compared to 311.002.2

The primer master mixes are delivered in solution. Extra markers are included in version 311.003-52 .

The number of tests in 311.003-52 are 52 and in 311.003-24 and 412.002-24 are 24. Extra markers included in the 311 kit will analyze 10 samples (313.003-10, 318.003-10, 321.003-10, 330.002-10).

One marker in solution 311.322 has been replaced: D13S256 has been replaced by D13S797

D13S797	13q33.2	13	Tetra	175-250	Blue

In the extra marker kit 313.003 we have replaced one marker (D13S252 with D13S325) and introduced one marker that is also available in the 311.003 kit (D13S305):

D13S325	13q14.11	13	Tetra	193*-231	Blue
D13S762	13q31.3	13	Tetra	270-326	Green
D13S305	13q13.3	13	Tetra	425-470	Green

Changed size limits

D18S976	18p11.31	18	Tetra	166-201	Yellow
D21S1409	21q21.2	21	Tetra	189-219	Yellow
D21S11	21q21.1	21	Tetra	224-270	Yellow
D21S1411	21q22.3	21	Tetra	283-344	Yellow
D21S1444*	21q22.13	21	Tetra	300-345*	Blue
DXS6854	Xq26.1	X	Tetra	91-119	Blue
D18S878	18q22.1	18	Tetra	153-192	Green

**this change is only valid for P/N 311.003*

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