



Frequently Asked Questions (FAQs) concerning ChromoQuant[®]:

Question 1: What is the ChromoQuant[®] prenatal diagnostic kit?

Answer 1: ChromoQuant[®] is the first CE-marked Quantitative Fluorescent (QF) PCR based prenatal diagnostic kit used in order to determine the chromosomal abnormalities such as: Trisomy 13 (Patau's Syndrome), Trisomy 18 (Edward's Syndrome), Trisomy 21 (Down 's syndrome), XXY-Klinefelter, XYY, XXX. The kit also identifies increased risk of X0-Turner 's syndrome.

The kit is also available as a version without the sex chromosome markers. See further information under Question 9.

Question 2: What are the principles used in ChromoQuant[®] prenatal diagnostic kit?

Answer 2: In the ChromoQuant[®] QF-PCR reaction, 4 STR markers are amplified in parallel in a multiplexed format for each chromosome (13, 18, 21 and X/Y), using fluorescently labelled PCR oligonucleotide primers. These primers are positioned adjacent to and surrounding the STR. Each specific STR on a given chromosome has a unique length with regard to the number of repeats it contains, thus discriminating one homologous chromosome from its counterpart in the somatic cell. The ideal normal, di-allelic, situation would indicate two separated peaks on a electropherogram whereas an ideal tri-allelic (trisomic) situation would indicate three separated peaks. However, homozygosity may occur.

Question 3: What are the advantages of using ChromoQuant[®] prenatal diagnostic kit?

Answer 3: The advantages of using ChromoQuant[®] prenatal diagnostic kit are as listed below:

- High sensitivity, reliability and accuracy as there is no false positive or false negative reported
- Result can be obtained within 24-48 hours, thus decreasing the anxiety of the parents
- Avoids detection of abnormalities of uncertain significance
- Suitable for high throughput analysis
- Sample pre-dispensed into PCR tubes
- Long term storage due to dry primer-mix
- Can be used for diagnosis of common chromosomal abnormalities in newborns, using mouth swabs
- CE marked kit is necessary for use in IVD applications within the EU
- CE mark guarantees production under controlled quality standards. In this case ISO13485-2003/ISO9001-2000

Question 4: What is included in a kit?

Answer 4: Each kit contains 1 unit of 300ul for enzyme dilution buffer, 1 unit of 1700ul of QF-PCR buffer and 96 PCR tubes with provided cover strips for 48 tests. There is no Taq Polymerase in the kit. Accordingly, the customer has to provide his /her own Taq Polymerase (see specification).

Question 5: How many samples can be analysed using one kit?

Answer 5: For each patient two tests have to be run, one each in order to analyse chromosome 13-18 and chromosome 21 respectively. Sex chromosome markers are included in both tests. Each kit has 96 PCR tubes. Thus, 48 tests for trisomy 13-18 and 48 tests for trisomy 21. Each kit will analyse 48 patients' samples.

Extra marker kits contain 48 samples for each chromosome, i.e. totally 96 samples can be analysed using the extra marker kits. Normally, just one extra marker set will be used for a separate sample to verify the status in a given chromosome.

Question 6: What is the starting DNA amount?

Answer 6: Each patient's DNA must run through two tests: trisomy 13-18 and trisomy 21 respectively. The minimum starting DNA amount for each PCR reaction should be 100-200 ng. Therefore, a minimum of 400 ng of DNA should be obtained from the amniotic fluid and make the final dilution of 10-20 ng μ L⁻¹ of DNA. In general, 2-3 ml of amniotic fluid is used for extraction of DNA.

Question 7: Do we need to get a license from Roche to use this kit?

Answer 7: The ChromoQuant[®] kit has IVD CE label and it does not include DNA Taq Polymerase. The customers have to provide their own Taq Polymerase. Two different enzymes are recommended.

GoTaq polymerase [®] Promega	P/N M8301 or M3171 (100U)
HotStar polymerase [®] Qiagen	P/N 203203 (100U)

Thus, this kit does not need a Taq-polymerase license from Roche for PCR. However, the customer must hold a licence from Roche in order to use the PCR technology for clinical purposes.



Question 8: Can we use any Taq polymerase on the market?

Answer 8: No! ChromoQuant[®] has been optimised for two enzymes:

GoTaq polymerase [®] Promega	P/N M8301 or M3171 (100U)
HotStar polymerase [®] Qiagen	P/N 203203 (100U)

Other enzymes have been tested but without reliable result.

Question 9: What products are available in the ChromoQuant[®] family?

Answer 9: Main kit for 48 samples	311.002-48u
Single tube test 48 samples	412.001-48u <i>without sex chr. markers</i>
Extra markers chr 13 and 18	631.001-48u
Extra markers chr 21, X/Y	651.001-48u

See further information under question 11, table 2.

Question 10: What are the requirements to run the ChromoQuant[®] QF-PCR based prenatal diagnosis kit?

Answer 10: In order to run the ChromoQuant[®] kit you must have access to a genetic analyzer from either Applied Biosystems or Amersham Biosciences/GE Healthcare (MegaBACE[®])

It depends on which genetic analyzer that is available in your lab. Please refer to table 1 below.

Table 1: Requirements to run the ChromoQuant® QF-PCR based prenatal diagnostic kit on genetic analyzers

Stages	Requirement	3100 Genetic Analyzer (3130 3730)	MegaBACE™ 500/1000	310 Genetic Analyzer (or ABI 377)
DNA Extraction	Sample	Amniotic Fluid	Amniotic Fluid	Amniotic Fluid
	DNA extraction kit	Common DNA extraction kit for amniotic fluid e.g. BioRad InstaGene Matrix or Qiagen EZ1	Common DNA extraction kit for amniotic fluid e.g. BioRad InstaGene Matrix or Qiagen EZ1	Common DNA extraction kit for amniotic fluid e.g. BioRad InstaGene Matrix or Qiagen EZ1
QF-PCR	ChromoQuant® Kit	311.002-48u 412.001-48u	311.002-48u 412.001-48u	311.002-48u (D-filter) 412.001-48u
	Taq Polymerase	Qiagen P/N 203203 Promega M3171, or M8301	Qiagen P/N 203203 Promega M3171, or M8301	Qiagen P/N 203203 Promega M3171, or M8301
Desalt or PCR-product purification	PCR-product purification kit	Common PCR-product purification kit	Common PCR-product purification kit	Common PCR-product purification kit
Running QF-PCR product on Genetic Analyzer	Matrix standard	Matrix standard set DS-30 (Applied Biosystems)	MegaBACE Long Read Matrix (GE Healthcare)	Flourescent Amidite Matrix standards (Applied Biosystems)
	Filter set	D filter (3100, 3130) Any 4 dye filter (3730)	Filter set 2	D filter (311 kits)
	Capillary	36 cm length, 50µ internal diameter, internally uncoated	40 cm length, outer diameter 200 um, inner diameter 75 um (standard)	Green labelled, 47 cm length, 50µ internal diameter, internally uncoated
	Polymer	POP4 (3100) POP7 (3130)	Linear Poly Acrylamid (LPA)	POP4
	Electrophoresis Size Standard	GeneScan-500 ROX	Amersham Biosciences ET-ROX or TAMRA	GeneScan-500 ROX (D-filter)
	Loading mix	12 µl HiDi FA + 0,3 µl size std + 0,5-1,0 µl PCR product	48µl water + 0,3 µl size std + 2 µl PCR product	12 µl HiDi FA + 0,3 µl size std + 0,5-1,0 µl PCR product
Result interpretation	Softwares	GeneScan, Gene Mapper or Genotyper, ChromoQuant Visualizer	Genetic Profiler or ChromoQuant Visualizer	GeneScan, Gene Mapper or Genotyper, ChromoQuant Visualizer

Other additional accessories	Pipette tips Thermocycler Centrifuge Spectrophotometer Genetic analyzer sampling tubes or plate
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Question 11: Which version of ChromoQuant[®] kit should we use?

Answer 11: ChromoQuant[®] has been designed for the use on two kinds of genetic analysers, ABI PRISM and Amersham Biosciences MegaBACE. Please refer to Table 2.

Table 2: Genetics Analyzer that involved in ChromoQuant[®] prenatal diagnosis

Manufacturer	Instrument models	ChromoQuant [®] kit
Applied Biosystems (ABI)	GA 310 (D-filter) GA 3100 GA 3100-A GA 3130 GA 3130 xl GA 3730 GA 3730 xl	311.002-48u 412.001-48u 631.001-48u 651.001-48u
	MegaBACE500 / MegaBACE1000	311.002-48u (filter set 2) 412.001-48u (filter set 2) 631.001-48u (filter set 1 or 2) 651.001-48u (filter set 1 or 2)

Question 12: How to interpret the results we get from the electropherogram?

Answer 12: Each genetic marker will generate a specific pattern in a well-defined area, in a predicted colour (green, yellow/ black, blue or red). Only heterozygote patterns can be used for correct interpretation. At least two markers for a particular chromosome must be heterozygotes in order to be used as diagnostic indication. Please refer to the **User's Guide** for detailed information and examples.

Question 13: What can we do if the results do not show at least two heterozygote markers in one chromosome?

Answer 13: You can run the extra markers for Chromosome 13, 18, 21 and X/Y (Part numbers; see above under Question 9).

Question 14: What are the limitations for ChromoQuant[®] QF-PCR based prenatal diagnosis kit?

Answer 14: This kit is not intended for evaluation or determination if a translocation has occurred, involving chromosome 13, 18, 21 or X/Y. However, assessing a trisomic state at chromosome level may still be possible. The kit must not be used in order to assess mosaic genetic aberrations. The kit will only give an indication about increased risk for Turner syndrome. If this risk is indicated, other methods such as FISH must be used in order to verify the status.

Question 15: What can we do if the amniotic fluid has been contaminated with maternal blood?

Answer 15: We recommend that another method is used in order to diagnose the sample.

Question 16: What if the two peaks in a heterozygote STR is not separated by more than two base pairs? Can this STR be used for diagnosing the sample?

Answer 16: This peak must not be used for diagnosing the sample. If necessary extra markers must be used in order to verify sample status.

Question 17: A sample contains one STR indicating trisomy (three peaks or two peaks with a 1:2 ratio). How should this sample be interpreted?

Answer 17: Extra markers must be used in order to verify sample status. Search in databases for further information concerning the actual marker. If possible genotypes should be run from parents DNA.

Question 18: Is desalting of the PCR product necessary?

Answer 18: Desalting might improve the signal – i.e. make it stronger. It might also reduce background noise. Normally, desalting is not necessary when using ABI instruments for electrophoresis.

Question 19: The signal of the peak exceeds the recommended value. Can this marker be used for interpretation of the result?

Answer 19: No, re-run the sample with shorter injection time or diluted PCR product.

Question 20: What method do you recommend for DNA purification

Answer 20: In order to purify DNA *from amniotic fluid* most users are using the InstaGene Matrix (BioRad #732-6030). Also EZ-1 (Qiagen #953034) has been tested with good result.

When it concerns purification of DNA from saliva we recommend the OraGene kit (DNA Genotek) and for DNA from mouth swabs the following products have been used: Buccal Amp DNA Extraction Kit, QuickExtract DNA Extraction Solution 1.0 and Catch-All, Sample Collection Swabs - (#BQ0901S, #QE09050, #QEC091H, Epicentre)