

AneuSure® Plus v2 kit is a QF-PCR kit for rapid detection of chromosomal aneuploidies for chromosomes 13, 18, 21, X and Y as well as detecting presence or absence of exon 7 of SMN1 and SMN2 genes for detecting SMA in every fetal sample which is tested for chromosomal aneuploidies.






With AneuSure® Plus v2 Kit, amplification of 29 markers in a multiplex PCR reaction is possible. All markers have been selected to have high heterozygosity and are distributed throughout the chromosomes 13, 18, 21, X, and Y. The kit also contains primers for quantifying exon 7 of SMN1 and SMN2 genes. Deletion of exon 7 of the SMN1 gene is seen to be deleted in 95-98% of 5q SMA cases. DNA template for AneuSure Plus v2 can be extracted from several sources: amniotic fluid (AF), chorionic villus (CVS), and blood. To prevent sample mix-up and to ensure the originality of the fetal sample, we recommend that the sample from the mother be amplified and used as a reference (please follow any national guidelines in your country). Another advantage of this kit is the reliable detection of Turner syndrome (Monosomy of X) by using 7/X and 11/X segmental duplication (SD) markers allowing the quantification of chromosome X as well as other sex chromosome markers.



Storage conditions

- Prevent exposure of primer mix to direct light. This may have an impact on the intensity of the fluorescent dye.
- Store all components at -20°C for better stability.
- Avoid repeated freezing-thawing cycles to maintain the good quality of the kit. We recommend aliquoting the kit components if are going to be used for a long duration.

AneuSure® Plus v2 kit components

Table 1: Provided with the Kit are Box A and Box B. They should be kept separately.

BOX-A		
	Tube Label	Tube cap color
1	PCR Mix	
2	Primer Mix	
3	GT HSTaq	
4	GT QCDF150(Control DNA-50ng/μl)	
5	GT QCW (H2O)	

BOX-B		
	Tube Label	Tube cap color
1	GT500 Size Standard	
2	GTM5 v2 (Optional)	

Instructions

1. Bring all components to room temperature.
2. Vortex Primer Mix and PCR Mix and spin down briefly to remove all residues from the lid. Gently mix the enzyme by inverting or pipetting.
3. Prepare a Master Mix for your reaction according to the following recipe. Every preparation can be done at room temperature. **Table 2: PCR reaction set-up**

Component	Volume for 1 reaction[μl]
GT QCW (H2O)	10
PCR Mix	7
Primer Mix	1
GT HSTaq	1

4. Vortex Master Mix briefly.
5. Transfer 19μl of Master Mix to each 0.2ml PCR tube for each sample you want to analyze.

6. Add 1µl of DNA template (5-10 ng per reaction) to each PCR tube.
7. Vortex and spin down each PCR tube content. Make sure that no droplets are left on the tube wall or lid.
8. Place tubes into a thermal cycler.
9. Please use the following PCR program to amplify all markers.
10. Store the PCR products at 2-6°C until analysis on a Genetic Analyzer.

Table 3: PCR program

Initial step	Cycling			Final Extension	Storing in Cycler
	Denaturation	Annealing	Extension		
95 °C	95 °C	63 °C	70 °C	70 °C	4 °C
20 min	1 min	90 sec	2 min	17-20 min	∞
27-30 Cycles					

Note

- We recommend storing PCR product at 2-6°C in a dark place (fluorescent dyes!).
- The quality of the results will be reduced with increasing time gap (about more than 2 weeks!) between PCR amplification and capillary electrophoresis.
 - A quality control (provided in the kit) and a negative control should be run in each Multiplex PCR to verify the successful amplification of each marker.
 - Varying quantities of DNA templates may require different numbers of cycles in the PCR program. Please see “AneuSure® Plus v2 User Manual” for further information.

WARNING

After PCR is complete, tubes should never be opened in the PCR setup area or beside kit components. Risk of contamination!

How to analyze data from the AneuSure® Plus v2 Kit

- AneuSure® Plus v2 Kit is optimized for use on Compact Spectrum CE System from Promega and ABI PRISM Genetic Analyzer like ABI 3130/xl or ABI3500/xL. Make sure your Data Collection Software supports 5-dye fragment analysis.
- Perform Spectral Calibration using GTM5 v2 Matrix Standard v2 (supplied with the kit).
- We recommend verifying a successful Multiplex PCR by gel electrophoresis before analyzing it on Genetic Analyzer (at least for the first time use or for troubleshooting).
- Prepare PCR products for capillary electrophoresis according to your CE system protocols. Analyze the samples using the AneuSure® Plus v2 GeneMapper Panel provided on our website.

Note

- For further information regarding the AneuSure® Plus v2 Kit please see “AneuSure® Plus v2 User Manual” (downloadable from the Genetek website). It includes recommendations for different DNA amounts per reaction, a table containing the names and sizes of all amplified markers as well as troubleshooting.
- To simplify the analysis of your samples, we have provided a panel.
- Please find all documents regarding the AneuSure Plus v2 Kit on our website.

www.genetek-biopharma.com.

- It may arise that alleles fall outside their size range and overlap with the size range of another locus. This appears at low frequencies in some populations.

- For any further clarification, please contact our technical service via email: (support@genetek.de).