

The GT HapScreen® F8 kit is a novel invention which is used to aid carrier detection or prenatal diagnosis of Hemophilia A as well as QF-PCR screening of fetal samples






The kit functions on the haplotyping/linkage principle using STR markers linked to the Factor VIII gene and QF-PCR technique. The kit components include primers for STR markers linked to the Factor VIII gene and our Aneasure QF-PCR kit in a single multiplex PCR. Factor VIII linked STR markers are designed from 5 regions that covers the upstream and downstream of the Factor VIII gene. Along with Hemophilia A detection it also screens for aneuploidies of chrs. 21, 18, 13, X and Y. This kit is optimized to be used on DNA samples purified from blood, amniotic fluid, and chorionic villus (CVS). This kit has many other advantages (please refer to GTHapScreen user manual and our website for more detailed information).



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.
- Avoid repeated freezing-thawing cycles to maintain the good quality of the kit. We recommend to aliquot the components if necessary.

GT HapScreen® F8 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 colour
1	PCR Mix	
2	Primer Mix	
3	GT HSTaq	
4	GT QCDM102(Control DNA-50ng/μl)	
5	GT QCW (H2O)	

BOX-B		
	Tube Label	Tube cap colour
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. Make sure that no droplets are left at the tube wall or lid.
8. Place tubes into thermal cycler.
9. Please use the following PCR program for the amplification of all markers.
10. Store the PCR products at 2-6°C until analysis with 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 reduce with increased time gap (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 successful amplification of each marker.
- Varying quantity of DNA template may require different numbers of cycles in PCR program. Please see “GT HapScreen® F8 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 GT HapScreen® F8 Kit

- GT HapScreen® F8 Kit is optimized for usage on ABI PRISM Genetic Analyzer like ABI 3130/xl or ABI3500/xL. Make sure your ABI Data Collection Software supports 5-dye fragment analysis.
- Perform Spectral Calibration using GTM5 v2 Matrix Standard (supplied by Genetek).
- We recommend verifying a successful Multiplex PCR by gel electrophoresis before analyzing it on Genetic Analyzer.
- Prepare PCR products for capillary electrophoresis according the ABI protocol. Analyze the samples using the GT HapScreen® F8 GeneMapper Panel provided on our website.

Note

- For further information regarding GT HapScreen® F8 Kit please see “GT HapScreen® F8 User Manual”. It includes recommendations for different DNA amounts per reaction, table containing the names and sizes of all amplified markers as well as troubleshooting.
- To simplify the analysis of your samples, we provide a panel.
- Please find all documents regarding GT HapScreen® F8 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).