

GT HapScreen® DMD

CAT# GT-11204

The GT HapScreen® DMD kit is a novel invention which is used to aid carrier detection or prenatal diagnosis of Duchene/ Becker Muscular Dystrophies as well as screening of fetal samples for aneuploidy via QF-PCR






The kit functions on the haplotyping/linkage principle. The kit components include primers for STR markers linked to 6 regions of the Dystrophin gene. These markers cover the upstream and downstream of the Dystrophin gene. Along with Duchene/ Becker Muscular Dystrophy detection it also screens for aneuploidies of chrs. 21, 18, 13, and X using some STRs markers from our Aneasure QF-PCR kit in a single multiplex PCR. This kit is optimized to be used on DNA samples purified from blood, amniotic fluid, and chorionic villus (CVS). This kit has many advantages including confirming direct method results, sample authenticity, ruling out/in MCC, etc. (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 upon receiving the kit.
- Avoid repeated freezing-thawing cycles to maintain the good quality of the kit. We recommend to aliquot the components if necessary.

GT HapScreen® DMD 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 QCDF150(Control DNA-50ng/μl)	
5	GT QCW (H2O)	

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

Instructions for use

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® DMD 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® DMD Kit

- GT HapScreen® DMD Kit is optimized for usage on ABI 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 Multiplex PCR by gel electrophoresis before analyzing it on Genetic Analyzer.
 - Prepare PCR products for capillary electrophoresis according the ABI recommendation.
- Analyze the samples using the GT HapScreen® DMD GeneMapper Panel provided on our website.

Note

- For further information regarding GT HapScreen® DMD Kit please see “GT HapScreen® DMD User Manual”. It includes recommendations for different DNA amounts per reaction, table containing 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 GT HapScreen® DMD Kit on our website: www.genetek-biopharma.com or ask for them by sending us email.
- 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 information, please contact our technical service via email: (support@genetek.de).