

Myotonic Dystrophy Type 1 CTG repeat detection kit







DM1 is caused by expansion of CTG trinucleotide repeat in the noncoding region of the DMPK gene. The GT DM1 Detector kit is designed to amplify a region on the DMPK gene containing the CTG repeat region. The kit design is as such that an affected individual will produce a normal peak and an expanded peak appears as redundant peaks. If the expanded peaks is less than 650 bp (about 175 repeats or fully affected with classical type) it will be visible. Normal individuals are either homozygote or heterozygote, but the repeat size will fall under 35 repeat range. Incorporation of bins in the panel will ease repeat calling.

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 MD1 Detector 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 QCDM102 (Control DNA-50ng/μl)	
5	GT QCDMD (Control DNA-50ng/μl)	
	GT QCW (H2O)	



BOX-B		
	Tube Label	Tube cap colour
1	GTE600 Size Standard	
2	GTM5 v2 (Optional)	

Table 2: PCR reaction set-up

Component	Volume for 1 reaction[μl]
GT QCW (H2O)	10
PCR Mix	10
Primer Mix	1
GT HSTaq	0.5

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.
4. Vortex Master Mix briefly.
5. Transfer 21.5μl of Master Mix to each 0.2ml PCR tube for each sample you want to analyze.
6. Add 1μl of DNA template (10-50 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	72 °C	72 °C	4 °C
20 min	1 min	70 sec	80 sec	15 min	∞
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

WARNING

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

- Two quality control DNA (one normal and an affected) are 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 MD1 Detector User Manual” for further information.

How to analyze data from GT MD1 Detector Kit

- GT MD1 Detector Kit is optimized for usage on ABI PRISM Genetic Analyzer like ABI 3130/xl or ABI3500/xL, SeqStudio, Spectrum Compact CE System. Make sure your ABI Data Collection Software supports 5-dye fragment analysis.
 - Perform Spectral Calibration using GTM5 v2 Matrix Standard (supplied by Genetek with the kit).
 - We recommend verifying a successful Multiplex PCR by agarose gel electrophoresis before analyzing it on Genetic Analyzer.
 - Prepare PCR products for capillary electrophoresis according the ABI protocol.
- Analyze the samples using the GT MD1 Detector GeneMapper Panel and bins as well as instruction provided in the user manual downloadable from Genetek website.

Note

- For further information regarding GT MD1 Detector Kit please see “GT MD1 Detector 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 have provided a panel with bins. Bins can easily convert size to allele. Therefore, there is no need for hand calculation.
- Please find all documents regarding GT MD1 Detector Kit on 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).

No.	Panel	Size Range	Disease severity
1	5-35 CTG	(124-215)	Normal
2	36-50 CTG	(218-260)	Presymptomatic
3	51-150 CTG	(263-559)	Classical
4	More than 150 CTG	(560-600)	Juvenile-Congenital

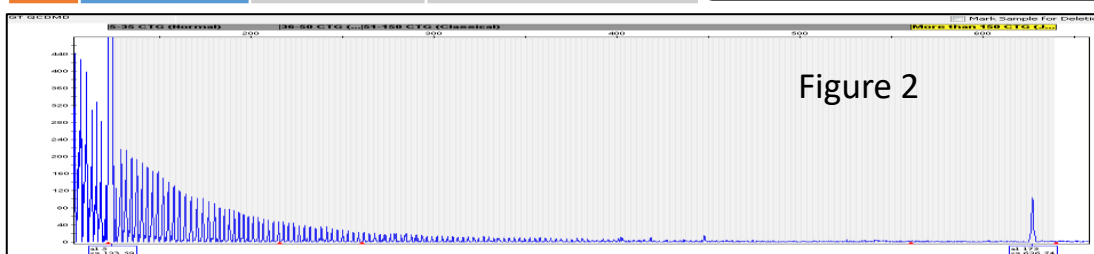
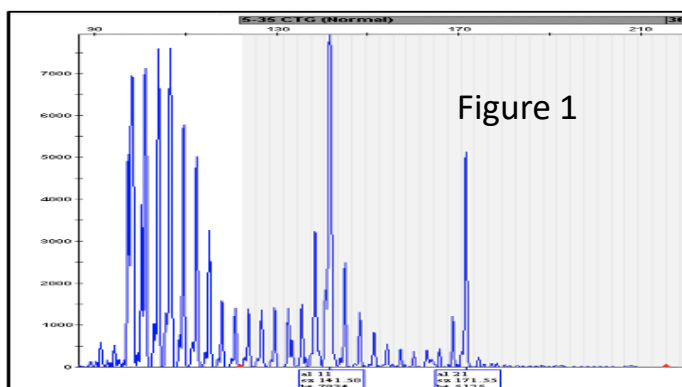


Figure 1. A heterozygote normal with 11 and 21 CTG repeats.
Figure 2. A heterozygote affected individual (Juvenile-Congenital) with 5 and 173 CTG repeats. The expanded repeats are usually in the range of GT 600 size standard included in the kit.