GeneQuery™ HLA-A26 Typing Kit by Melt Curve Analysis (GTM-HA26)

Catalog #HA26

250 reactions

Product Description

The human leukocyte antigen (HLA) complex is a gene complex located on the short arm of human chromosome 6. The human version of the major histocompatibility complex (MHC) helps the immune system distinguish the host cells from invaders. It is the most polymorphic locus in the human genome, and has been implicated in many pathologies including autoimmunity disorders and cancer.

GeneQuery™ HLA-A26 Typing Kit by Melt Curve Analysis (GTM-HA26) utilizes SYBR® Green or a similar fluorescent dye to reveal the presence or absence of HLA-A26 allele in a human genomic DNA (gDNA) sample. Following qPCR with melt curve analysis, the melt temperature (T_m) of the qPCR product is compared to a predetermined threshold temperature ($T_{threshold}$). A T_m higher than $T_{threshold}$ suggests the presence of the target allele, and a T_m lower than $T_{threshold}$ suggests the absence of the target allele (see Figure 1 and Table 1 for an example). For GeneQuery™ HLA-A26 Typing Kit by Melt Curve Analysis (GTM-HA26), the $T_{threshold}$ is 87.8°C (see Table 4).

Each GeneQuery™ HLA typing kit contains a positive Genomic DNA (gDNA) Control (GDC). It contains a primer set targeting a region on human chromosome 3. The GDC is included to examine the quality of gDNA sample, qPCR components and qPCR conditions.

The carefully designed primers included in GTM-HA26 ensure that: (i) the optimal primer annealing temperature in qPCR analysis is 65°C (with 2 mM Mg^{2+}, and no DMSO); (ii) no primer dimer formation under the recommended qPCR conditions.; and (iii) the difference in T_m of qPCR products when HLA-A26 is present or absent is greater than 1°C, which can be easily recognized by melt curve analysis or high resolution melt curve analysis.

Kit Components

Cat #	Component	Quantity	Storage
HA26a	HLA-A26 primer set, lyophilized	1 vial	-20°C
HA00b	Human genomic DNA control (GDC), lyophilized	1 vial	-20°C
HA00c	Nuclease-free H_2O	10 mL	4°C
HA00d	SYBR Green master mix	5 mL	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
genomic DNA template	Customers' samples
qPCR plate or tube	qPCR machine dependent

Figure 1. A typical melt curve analysis graph of an HLA typing assay.

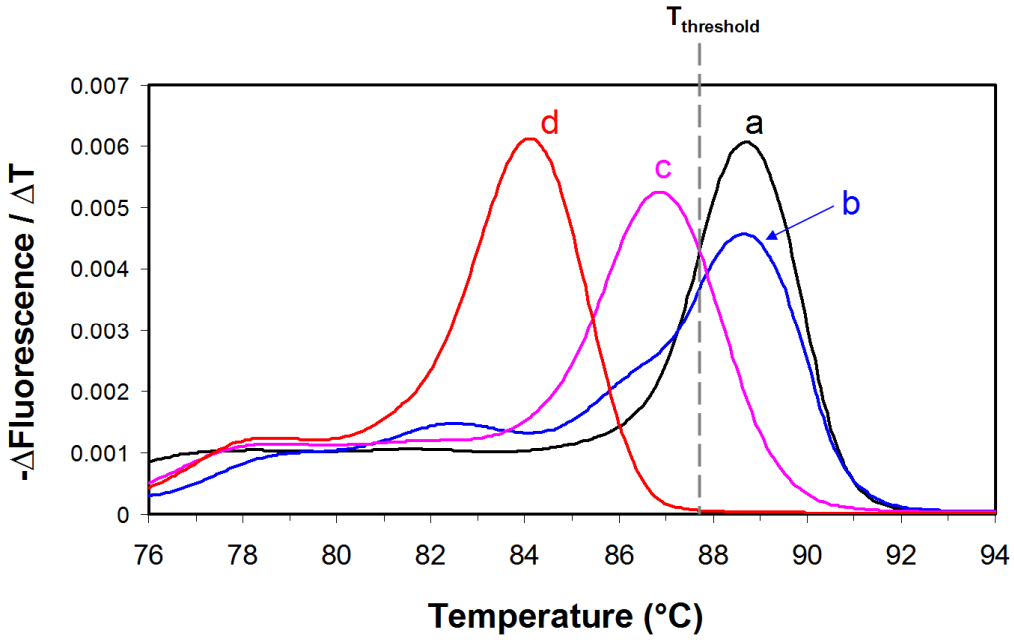

Table 1.

gDNA sample	Sample alleles*	T_m (°C)
a	Y / Y	88.69
b	Y / N_1	88.65
c	N_2 / N_3	86.86
d	N_4 / N_5	84.11

* For sample alleles, Y indicates the allele in the sample is the target allele of the kit, N_1 through N_5 are 5 distinct alleles that are not the target allele of the kit.

Quality Control

The specificity of the primer sets are validated by qPCR with melt curve analysis. The PCR products are analyzed by gel electrophoresis.

Product Use

GTM-HA26 is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, store the primers and SYBR Green master mix at -20°C in a manual defrost freezer, and nuclease-free H_2O at 4°C. Always keep SYBR Green master mix from light. Once SYBR Green master mix is thawed, store it at 4°C and do not refreeze. SYBR Green master mix is stable at 4°C for up to 6 months if stored properly.

Procedures

***Important: Only** use nuclease-free reagents in PCR amplification.*

Note: GTM-HA26 is optimized using SYBR Green master mix provided in the kit (Cat #HA00d). Use of other qPCR master mixes may compromise results.

1. Prior to use, allow vials (Cat #HA26a and #HA26b) to warm to room temperature.
2. Centrifuge the vials at 1,500x g for 1 minute.
3. Add 500 µl nuclease-free H_2O (Cat #HA00c) to HLA-A26 primer set (lyophilized, Cat #HA26a) to make HLA-A26 primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
4. Add 500 µl nuclease-free H_2O (Cat #HA00c) to GDC primer set (lyophilized, Cat #HA00b) to make GDC primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
5. For each genomic DNA sample, prepare two 20 µl qPCR reactions, one with HLA-A26 primer stock solution, and one with GDC primer stock solution, as shown in Table 2.

Table 2.

Genomic DNA template	0.5 – 20 ng
Primer stock solution (HLA-A26 or GDC)	2 µl
2x qPCR master mix (Cat #HA00d)	10 µl
Nuclease-free H_2O (Cat #HA00c)	variable
Total volume	***20 µl***

6. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds. For maximum reliability, replicates are strongly recommended (minimum of 3).
7. For qPCR program setup, refer to the instruction of the master mix of user's choice. We recommend a 2-step qPCR protocol with melt curve analysis as shown in Table 3:

Table 3.

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	40
Annealing / Extension	72°C	30 sec	
Data acquisition	Plate read		
T_m calling	Melt curve analysis or high resolution melt curve analysis		1
Hold	25°C	Indefinite	1

8. Refer to the instruction of the qPCR program of user's choice to obtain the T_m of the samples, and refer to Table 4 to determine the presence or absence of HLA-A26 in the samples.

Table 4. Interpretation of results:

Results	Interpretation
T_m of HLA-A26 qPCR product above 87.8°C	Allele HLA-A26 is ***Present***
T_m of HLA-A26 qPCR product below 87.8°C	Allele HLA-A26 is ***Absent***
No amplification of HLA-A26 qPCR, while GDC qPCR is positive	Allele HLA-A26 is ***Absent***
No amplification of both HLA-A26 and GDC qPCR	Defective qPCR conditions, poor gDNA sample quality, or gDNA amount below detection limit