Rev.0

GeneQuery™ HLA-A*02 Screening Kit by qPCR (GSHA02)

Catalog #GK830

50 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.

ScienCell's GeneQuery™ HLA-A*02 Screening Kit by qPCR (GSHA02) provides a convenient and fast way for HLA-A*02 allele screening with greater than 99% accuracy rate. GSHA02 utilizes SYBR®Green-based qPCR with melt curve analysis to reveal the presence or absence of HLA-A*02 allele in a human cell sample. GSHA02 can utilize either cell lysate or purified genomic DNA as the qPCR template. The cell lysis buffer and enhancer (Cat #GQ400a and GQ400b) are included in the kit to lyse pelleted cell samples. Each GSHA02 kit contains a HLA-A*02 (A02) primer set and a genomic DNA Control (HC21f) primer set. The A02 primer set amplifies only the HLA-A*02 allele, but not other HLA-A alleles. The HC21f primer set targets a region on human chromosome 21 and is included to examine the quality of the test sample, qPCR components, and qPCR conditions. Each primer set included in GSHA02 has been validated by qPCR with melt curve analysis and gel electrophoresis for amplification specificity. The 2x GoldNStart TaqGreen qPCR Master Mix (Cat #MB6018a-1) is a SYBR®Green dye-based qPCR master mix with a "hot-start" property. It contains SYBR®Green, dNTPs, Taq DNA polymerase, and an inert gold-color loading indicator in a single tube. The "hot-start" property achieved through ScienCell's unique chemically modified Taq DNA polymerase provides maximal inhibition of primer dimer formation. The advanced buffer formulation provides superior specificity and efficiency with a wide linear dynamic range. The inert gold-color loading indicator allows for better visualization and tracking of sample loading in qPCR plates or tubes. The HLA-A*02 positive genomic DNA control (Cat #GK830b) is the gDNA extracted from HLA-A*02 positive cells.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	2x GoldNStart TaqGreen qPCR master mix	1 mL	-20°C
GQ400a	Cell lysis buffer	10 mL	4°C
GQ400b	Cell lysis buffer enhancer, 100x	100 µL	-20°C
GK830a	HLA-A*02 (A02) primer set, lyophilized, 50 reactions	1 vial	-20°C
HC21f	Human genomic DNA control (HC21f) primer set, lyophilized, 50 reactions	1 vial	-20°C
GK830b	Genomic DNA control, HLA-A*02 positive, ~10 ng/µL	20 µL	-20°C
GQ100-04	Nuclease-free H_2O	4 mL	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
Cell pellets	Customers' samples
Heat blocks	with upper temperature limit above 95°C
qPCR instrument	
qPCR plates or tubes	qPCR instrument-dependent
gDNA extraction kit ***(optional)***	SpeeDNA Isolation Kit (ScienCell, Cat #MB6918)

Quality Control

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

Product Use

GSHA02 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 (Cat #GK830a and #HC21f), the cell lysis buffer enhancer (Cat #GQ400b), the positive gDNA control (Cat #GK830b), and GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) at -20°C in a manual defrost freezer. Store the cell lysis buffer (Cat #GQ400a) and nuclease-free H_2O (Cat #GQ100-04) at 4°C. Always keep GoldNStart TaqGreen qPCR master mix from light. Once GoldNStart TaqGreen qPCR master mix is thawed, store it at 4°C and do not refreeze. GoldNStart TaqGreen qPCR master mix is stable at 4°C for up to 6 months if stored properly.

Procedures

***Important: Only** use polymerases with hot-start capability to prevent possible primer-dimer formation. **Only** use nuclease-free reagents in PCR amplification.*

A. Preparation of cell lysate samples

Note: Skip Section A if using purified genomic DNA as the qPCR template.

1. For each sample, count the number of cells to be harvested. Harvesting 0.1-0.4 million cells per sample is recommended. Wash cells with PBS once, pellet cells and carefully remove PBS.

2. Determine the total volume of cell lysis buffer (Cat #GQ400a) to be used for the samples at 2,000 cells/µL cell lysis buffer. Transfer the calculated amount of cell lysis buffer with 5% extra to a new tube. Supplement the aliquoted cell lysis buffer with cell lysis buffer enhancer (100x, Cat #GQ400b). For every milliliter of cell lysis buffer, add 10 µL of cell lysis buffer enhancer (see an "example of calculations" below).

3. Transfer the supplemented cell lysis buffer to each cell pellet sample at 2,000 cells/µL supplemented cell lysis buffer. Carefully pipette the cell pellet up and down 20 times without generating bubbles. The samples should be homogenous. If not, continue pipetting until fully homogenized.

4. Incubate the homogenized samples at 55°C for 30 minutes, followed by incubating at 95°C for 10 minutes to fully lyse the samples. Alternatively, transfer 20 µL of each homogenized sample from step A.3 to a PCR tube, and run a PCR program as shown in Table 1.

Table 1. PCR program settings for lysing the cells

Step	Temperature	Time	Number of cycles
1	55°C	30 min	1
2	95°C	10 min	1
Hold	4°C	Indefinite	1

Example of calculations: Sample A has 0.1 million cells and sample B has 0.4 million cells.

In step **A.2**, aliquot (0.1 + 0.4) x 10^6/ 2,000 x 105% µL = 263 µL of cell lysis buffer (Cat #GQ400a), then add 263 µL x 10 µL/1 mL = 2.63 µL of cell lysis buffer enhancer (100x, Cat #GQ400b) to the aliquoted cell lysis buffer.

In step **A.3**, transfer 0.1 x 10^6/ 2,000 = 50 µL of supplemented cell lysis buffer to sample A, and 0.4 x 10^6/ 2,000 = 200 µL of supplemented cell lysis buffer to sample B.

5. Spin the samples at 1,000 rpm for 10 seconds at room temperature.

6. Keep lysed samples on ice or store at -20°C. Samples can be stored at -20°C for up to 12 months.

B. qPCR reaction setup

1. When using this kit for the first time, allow primer set vials (Cat #GK830a and #HC21f) to warm to room temperature.

2. Centrifuge the vials at 1,500x g for 1 minute.

3. Add 100 µL nuclease-free H_2O (Cat #GQ100-04) to A02 primer set (lyophilized, Cat #GK830a) to make A02 stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

4. Add 100 µL nuclease-free H_2O (Cat #GQ100-04) to HC21f primer set (lyophilized, Cat #HC21f) to make HC21f stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

5. For each sample, prepare two PCR reactions, one with A02 primer stock solution, and one with HC21f primer stock solution. Prepare 20 µl qPCR reaction per well as shown in Table 2.

6. For HeLa Positive control PCR template (Cat #GK830b), prepare two PCR reactions, one with A02 primer stock solution, and one with HC21f primer stock solution. Prepare 20 µl qPCR reaction per well as shown in Table 2.

7. Prepare two "no-template" negative control PCR reactions by using H_2O as the PCR template, one with A02 primer stock solution, and one with HC21f primer stock solution. Prepare 20 µl qPCR reaction per well as shown in Table 2.

   Note: When using purified genomic DNA as the template, dilute the gDNA to 10 ng/µL, and take 1 µL for each qPCR reaction.

   Table 2. qPCR reaction preparation

Template: lysed cell sample, gDNA sample, positive control (Cat #GK830b), or H_2O	1 µL
Primer stock solution (A02 or HC21f)	2 µL
2x GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µL
Nuclease-free H_2O (Cat #GQ100-4)	7 µL
Total volume	***20 µL***

8. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds.

9. Refer to Table 3 for qPCR program setup. The 2x GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) contains SYBR®Green as the reporter dye and does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.

   Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of A02 primer set (Cat #GK830a) and HC21f primer set (Cat #HC21f), we highly recommend an annealing temperature of 64°C as shown in Table 2:

Table 3. Recommended qPCR program

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	35
Annealing	64°C	20 sec	
Extension	72°C	30 sec	
Data acquisition	Plate read		
T_m calling	*Melting curve analysis*		1
Hold	20°C	Indefinite	1

C. Results interpretation

1. $Cq_{(HC21f)}$ is the quantification cycle value obtained from the qPCR program using the HC21f primer set. Any sample with a $Cq_{(HC21f)}$ value higher than 27 is considered assay failed. The possible reasons include substandard sample quality, low-quality qPCR components, or wrong qPCR conditions. In this case, do NOT call the HLA-A*02 status of the sample.

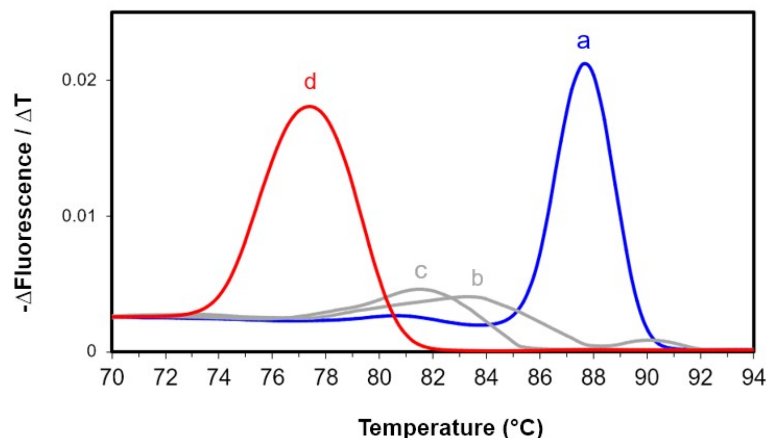
2. $Cq_{(A02)}$ is the quantification cycle value obtained from the qPCR program using the A02 primer set. $\Delta Cq_{(sample)}$ is the quantification cycle value difference between $Cq_{(A02,\ sample)}$ and $Cq_{(HC21f,\ sample)}$. If $\Delta Cq_{(sample)} > 4$, then this sample is HLA-A*02 negative.

$$\Delta Cq_{(sample\ 1)} = Cq_{(A02,\ sample\ 1)} - Cq_{(HC21f,\ sample\ 1)}$$

3. When $Cq_{(HC21f)} < 27$ and $\Delta Cq_{(sample)} < 4$, check the melting curves of the qPCR reaction products. An HLA-A*02 positive sample shows a single distinct peak with A02 primer set at 87.5°C and a single distinct peak with HC21f primer set at 77.5°C (Figure 1).

Figure 1. A typical melt curve analysis graph of an HLA-A*02 screening assay.

a) the melting peak of an HLA-A*02 positive sample with A02 primer set; $Tm_{(A02)} = 87.5$°C;
b) the melting peak of an HLA-A*02 negative sample with A02 primer set;
c) the melting peak of an HLA-A*02 negative sample with A02 primer set;
d) the melting peak of an HLA-A*02 positive sample with HC21f primer set; $Tm_{(HC21f)} =$ 77.5°C.



4. The summary of results interpretation is listed in Table 4.

Table 4.

Results		Interpretation
$Cq_{(HC21f)} > 27$		Assay Failed
$Cq_{(HC21f)} < 27$; and $\Delta Cq_{(sample)} > 4$		HLA-A*02 negative
$Cq_{(HC21f)} < 27$; and $\Delta Cq_{(sample)} < 4$	87°C < $Tm_{(A02)}$ < 88°C	HLA-A*02 positive
	$Tm_{(A02)}$ < 86°C	HLA-A*02 negative