Rev.1

GeneQuery™ Human Biological Sex Determination qPCR Assay Kit (HSDQ)

Catalog #GK820

50 reactions

Product Description

Biological sex is genetically intrinsic to every cell of a sexually reproducing organism, and sex-differentiated characteristics can be found in many complex human phenotypes, including diseases, that influence tissue and cell experiments. The sex of tissues or cells can also be important to note when performing experiments in order to aid in result reproducibility or ensure that findings are not over-generalized. There are occasions, however, when the sex of a sample is unknown and it is crucial to differentiate biological male tissue from biological female tissue.

ScienCell's Human GeneQuery™ Biological Sex Determination qPCR Assay Kit (HSDQ) is designed to determine whether a sample of unknown sex is derived from biologically male or female tissue. The Male-specific primer set recognizes only the male, but not the female. The sex chromosome primer set recognizes both male and female genetic material and serves as an indicator of genetic material in the testing samples. The two reference genomic DNA samples provide a reference by providing one biologically male reference and one biologically female reference. The carefully designed primers ensure: (i) high efficiency for trustworthy quantification; and (ii) no non-specific amplification. Each primer set has been validated by qPCR with melt curve analysis and gel electrophoresis for amplification specificity and by template serial dilution for amplification efficiency.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	2X GoldNStart TaqGreen qPCR master mix, 1 mL	2 vials	-20°C
GQ400a	Cell lysis buffer	20 mL	4°C
GQ400b	Cell lysis buffer enhancer, 100x	200 µL	-20°C
GK820a	Male-specific primer set (MSP), lyophilized, 50 reactions	1 vial	-20°C
GK820b	Sex chromosome primer set (SCP), lyophilized, 50 reactions	1 vial	-20°C
GK820c	Female genomic DNA control, ~10 ng/µL	20 µL	-20°C
GK820d	Male genomic DNA control, ~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
Heat blocks	With upper temperature limit above 95°C
qPCR plate or tube	qPCR machine 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
GK820 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, the cell lysis buffer enhancer, the gDNA controls, and SYBR Green master mix at -20°C in a manual defrost freezer. Store the cell lysis buffer and nuclease-free H2O 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 polymerases with hot-start capability to prevent possible primer-dimer formation.* ***Only *use nuclease-free reagents in PCR amplification.*

A. Preparation of cell lysate

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 0.2-0.8 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 (see an "example of calculations" below). 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.

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. Keep lysed samples on ice or store at -20˚C.

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 0.4 million cells and sample B has 0.8 million cells.

In step **A.2**, aliquot (0.4 + 0.8) x 10^6 / 2,000 x 105% µL = 630 µL of cell lysis buffer (Cat #GQ400a), then add 630 µL x 10 µL/1 mL = 6.30 µL of cell lysis buffer enhancer (100x, Cat #GQ400b) to the aliquoted cell lysis buffer.

In step **A.3**, transfer 0.4 x 10^6 / 2,000 = 200 µL of supplemented cell lysis buffer to sample A, and 0.8 x 10^6 / 2,000 = 400 µL of supplemented cell lysis buffer to sample B.

B. qPCR reaction setup

1. When using this kit for the first time, allow the MSP primer set vial (lyophilized, Cat #GK820a) and the SCP primer set vial (lyophilized, Cat #GK820b) to warm to room temperature.

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

3. Add 100 µL nuclease-free H2O (Cat #GQ100-04) to MSP primer set (lyophilized, Cat #GK820a) to make MSP 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 H2O (Cat #GQ100-04) to SCP primer set (lyophilized, Cat #GK820b) to make SCP stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

5. Prepare qPCR reactions with MSP stock solution and SCP stock solution for the lysed cell samples. Prepare 20 µL qPCR reactions as shown in Table 2. Set up two control qPCR reactions with female genomic DNA control (Cat #GK820c) and male genomic DNA control (Cat #GK820d) as the template each, and one "no-template" negative control qPCR reaction by adding H_2O as the template.

   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 setup for HLA-A*02 screening

Template: lysed cell sample, gDNA sample, controls (Cat #GK820c and GK820d), or H_2O	1 µl
Primer stock solution (MSP or SCP)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #GQ100-04)	7 µl
Total volume	***20 µl***

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

7. 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 MSP primer set (Cat #GK820a) and SCP primer set (Cat #GK820b), we highly recommend an annealing temperature of 65°C as shown in Table 2:

   Table 3. qPCR program settings for biological sex determination

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	35
Annealing	65°C	20 sec	
Extension	72°C	30 sec	
Data acquisition	Plate read		
Tm calling	*Melting curve analysis*		1
Hold	20°C	Indefinite	1

C. Results interpretation

1. $Cq_{(SCP)}$ is the quantification cycle value obtained from the qPCR program using the SCP primer set. Any sample with a $Cq_{(SCP)}$ 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 biological sex of the sample.

2. $Cq_{(MSP)}$ is the quantification cycle value obtained from the qPCR program using the MSP primer set. $\Delta Cq_{(sample)}$ is the quantification cycle value difference between $Cq_{(MSP, sample)}$ and $Cq_{(SCP, sample)}$. If $Cq_{(MSP, sample)}$ shows no value due to lack of PCR amplification, or $\Delta Cq_{(sample)} > 4$, then this sample is biologically female. If $\Delta Cq_{(sample)} < 4$, then this sample is biologically male.

$$\Delta Cq_{(sample\ 1)} = Cq_{(MSP,\ sample\ 1)} - Cq_{(SCP,\ sample\ 1)}$$

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

Table 4.

Results	Interpretation
Cq (SCP) > 27	Assay Failed
Cq (SCP) < 27; and MSP shows no PCR amplification	Female
$Cq_{(SCP)} < 27$; and $\Delta Cq_{(sample)} > 4$	Female
$Cq_{(SCP)} < 27$; and $\Delta Cq_{(sample)} < 4$	Male