Absolute Human Kringle Repeats Quantification qPCR Assay Kit (AHKRQ)

Catalog #GK801
100 reactions

Product Description

Lipoprotein(a), or Lp(a), is one subclass of lipoproteins. It is the defining protein component of Lp(a) particles. Plasma Lp(a) concentrations have been associated with atherosclerotic and cardiovascular diseases. It has been reported that plasma Lp(a) concentrations are hereditary and have been linked to the highly polymorphic LPA gene located on human chromosome 6. LPA gene contains 10 types of kringles, namely kringle-IV types 1-10 (KIV1-10), that are highly homologous in sequence. Moreover, one type of kringle, KIV2, exhibits a high level of copy number variation. The number of KIV2 repeats can vary from 5 to over 50 among human populations.

ScienCell's Absolute Human Kringle Repeats Quantification qPCR Assay Kit (AHKRQ) is designed to directly measure the number of kringle repeats of a human cell sample. The kringle repeats (KRG) primer set recognizes and amplifies all kringle IV repeats, including KIV1-10. The single copy reference (SCR) primer set recognizes and amplifies a region on human chromosome 17, and serves as reference for data normalization. The reference DNA sample with a 1:1 ratio of kringle repeats copies to SCR copies serves as a reference for calculating the number of kringle repeats of target samples. 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
GK801a	Kringle repeats (KRG) primer set, lyophilized	1 vial	-20°C
GK801b	Single copy reference (SCR) primer set, lyophilized	1 vial	-20°C
GK801c	Nuclease-free H_2O	4 mL	4°C
GK801d	Reference DNA sample	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
genomic DNA template	Customers' samples
qPCR plate or tube	
qPCR master mix	FastStart Essential DNA Green Master (Roche, Cat #06402712001)

Quality Control

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

template serial dilution (See **Appendices 1 and 2**). The reference genomic DNA sample is sequenced by Sanger sequencing.

Product Use

AHKRQ 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 #GK801a and GK801b) and the reference genomic DNA sample (Cat #GK801d) at -20°C in a manual defrost freezer, and nuclease-free H_2O (Cat #GK801c) at 4°C.

Procedures

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

Note: The quality of the qPCR master mix is a critical element for successful qPCR analyses. AHKRQ is optimized using FastStart Essential DNA Green Master (Roche, Cat #06402712001) and is highly recommended. Use of other qPCR master mixes may compromise results.

1. Prior to use, allow vials (Cat #GK801a and #GK801b) to warm to room temperature.

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

3. Add 200 µl nuclease-free H_2O (Cat #GK801c) to KRG primer set (lyophilized, Cat #GK801a) to make KRG primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

4. Add 200 µl nuclease-free H2O (Cat #GK801c) to SCR primer set (lyophilized, Cat #GK801b) to make SCR primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

5. For the reference DNA sample (Cat #GK801d), prepare two qPCR reactions, one with KRG primer stock solution, and one with SCR primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 1.

Table 1.

Reference DNA sample (Cat #GK801d)	1 µl
Primer stock solution (KRG or SCR)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #GK801c)	7 µl
Total volume	***20 µl***

6. For each genomic DNA sample, prepare two qPCR reactions, one with KRG primer stock solution, and one with SCR primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 2.

Table 2.

Genomic DNA template	5 ng
Primer stock solution (KRG or SCR)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #GK801c)	variable
Total volume	***20 µl***

7. 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).

8. For qPCR program setup, refer to Table 3 when using FastStart Essential DNA Green Master (Roche, Cat #06402712001). This master mix 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. When using other qPCR master mixes, the qPCR program may require optimization with Table 3 as a starting protocol.

Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of KRG and SCR primer sets (Cat #GK801a and #GK801b), we highly recommend an annealing temperature of 60°C 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	35
Annealing	60°C	20 sec	
Extension	72°C	20 sec	
Data acquisition	Plate read		
Optional	*Melting curve analysis*		1
Hold	20°C	Indefinite	1

Figure 1. A typical amplification curve showing the amplification of a qPCR product.

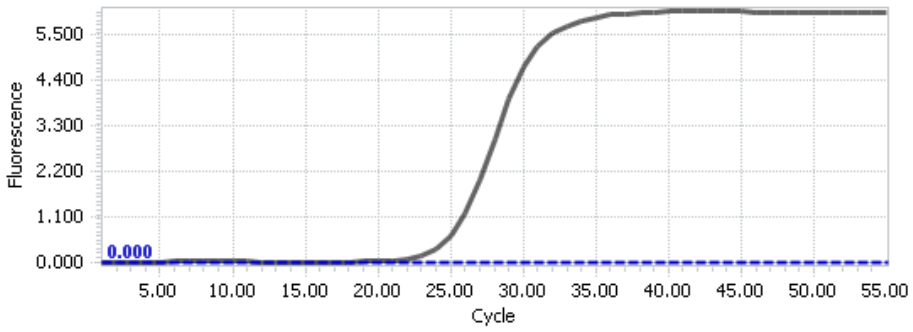
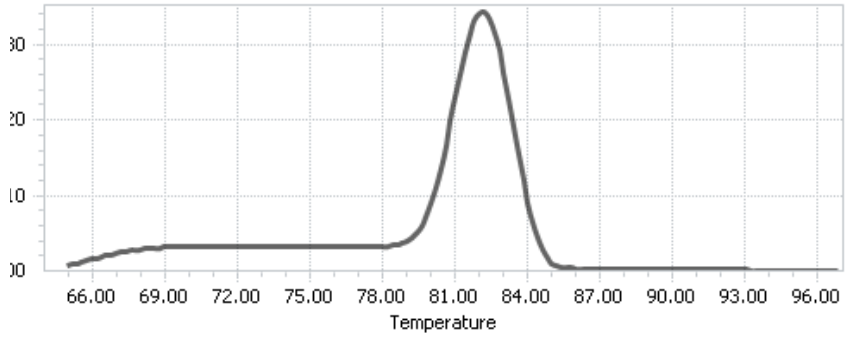


Figure 2. A typical melting peak of a qPCR product.



Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

Note: Please refer to your qPCR instrument's data analysis software for data analysis. The method provided here serves as guidance for quick manual calculations.

1. For KRG (KRG), ΔCq (KRG) is the quantification cycle number difference of KRG between the target and the reference DNA samples.

   ΔCq (KRG) = Cq (KRG, target sample) - Cq (KRG, reference sample)

   Note: the value of ΔCq (KRG) can be positive, 0, or negative.

2. For single copy reference (SCR), ΔCq (SCR) is the quantification cycle number difference of SCR between the target and the reference DNA samples.

   ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)

   Note: the value of ΔCq (SCR) can be positive, 0, or negative.

3. ΔΔCq = ΔCq (KRG) - ΔCq (SCR)

4. The ratio of kringle repeats to SCR of the target sample = $2^{-\Delta\Delta Cq}$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 3. Cq (Quantification Cycle) values of KRG qPCR (KRG) and single copy reference qPCR (SCR) obtained for the samples.

Primer set	*Target sample*	*Reference sample*
KRG	21.08	20.99
SCR	25.64	20.80

ΔCq (KRG) = Cq (KRG, target sample) - Cq (KRG, reference sample)
= 21.08 - 20.99
= 0.09

ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)
= 25.64 - 20.80
= 4.84

ΔΔCq = ΔCq (KRG) - ΔCq (SCR)
= 0.09- (4.84)
= -4.75

The ratio of kringle repeats to SCR of the target sample

$= 2^{-\Delta\Delta Cq}$

$= 2^{4.75}$

$= 27$

Conclusions: There are 2 alleles of LPA gene and 2 copies of SCR in a diploid cell. The average number of kringle repeats of the 2 alleles of target sample is 27.

Appendix 1: Quality assessment of kringle repeats (KRG) primer set

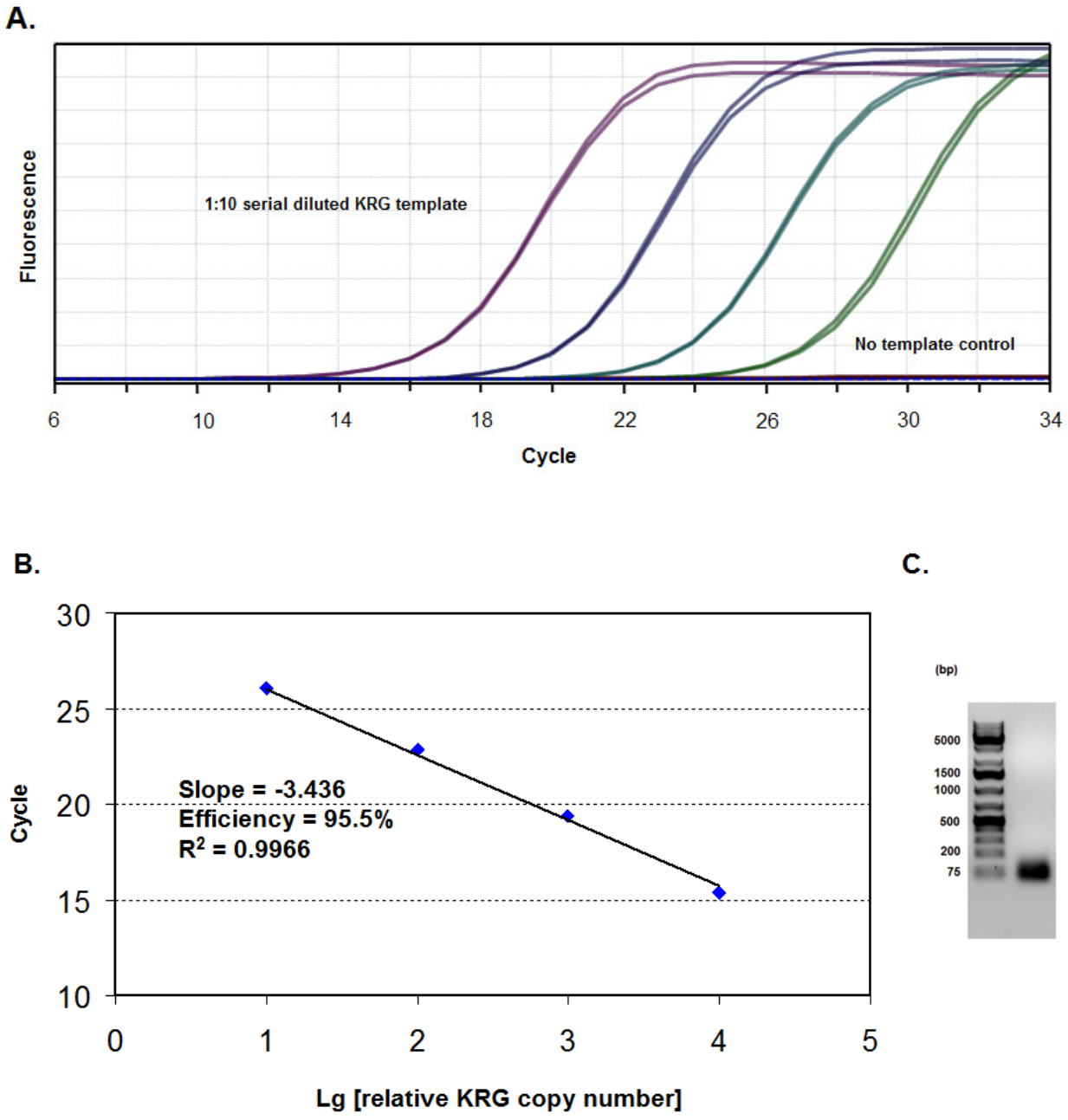


Figure 3. Quality assessment of KRG primer set. **(A)** qPCR amplification curves using serially diluted KRG repeats as template. **(B)** Derivation of qPCR efficiency of KRG primer set. **(C)** Separation of KRG qPCR product by gel electrophoresis.

Appendix 2: Quality assessment of single copy reference (SCR) primer set

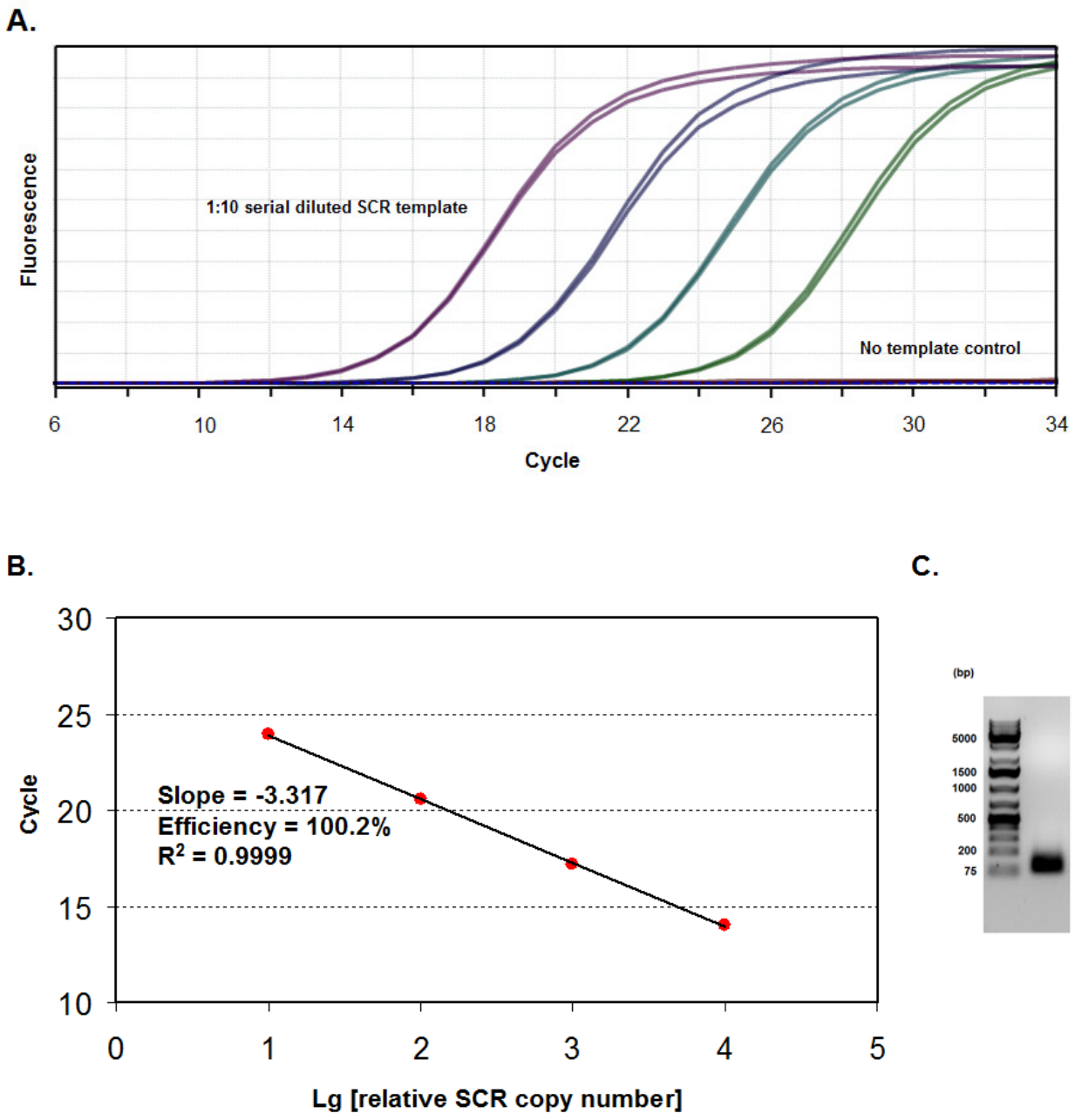


Figure 4. Quality assessment of Single copy reference (SCR) primer set. **(A)** qPCR amplification curves using serially diluted SCR template. **(B)** Derivation of qPCR efficiency of SCR primer set. **(C)** Separation of SCR qPCR product by gel electrophoresis.