PrimePCR™ Assay Validation Report

Gene Information

Gene Name	pyruvate kinase, liver and RBC
Gene Symbol	PKLR
Organism	Human
Gene Summary	The protein encoded by this gene is a pyruvate kinase that catalyzes the transphosphorylation of phohsphoenolpyruvate into pyruvate and ATP which is the rate-limiting step of glycolysis. Defects in this enzyme due to gene mutations or genetic variations are the common cause of chronic hereditary nonspherocytic hemolytic anemia (CNSHA or HNSHA). Multiple transcript variants encoding different isoforms have been found for this gene.
Gene Aliases	PK1, PKL, PKR, PKRL, RPK
RefSeq Accession No.	NC_000001.10, NG_011677.1, NT_004487.19, NW_003315906.1
UniGene ID	Hs.95990
Ensembl Gene ID	ENSG00000143627
Entrez Gene ID	5313

Assay Information

Unique Assay ID	qHsaCEP0052411
Assay Type	Probe - Validation information is for the primer pair using SYBR® Green detection
Detected Coding Transcript(s)	ENST00000329021, ENST00000231721, ENST00000270233, ENST00000591949, ENST00000304613, ENST00000368571, ENST00000368572, ENST00000392414, ENST00000342741, ENST00000368539, ENST00000480071, ENST00000357296, ENST00000278060, ENST00000426431, ENST00000572740, ENST00000571194
Amplicon Context Sequence	TCAGATAGGCCTCAGGTAGGGAGGGTCAGGAATAGAGAAGAGAGGACTTAAAG GTGGGGCTTTGGAGGGGTGTGGGCTGGAGAACGTAGACTGGGGAGGAAGGGA TGGGGTACAAGGGTAGGCTGGGCCAGAGGAGGGAGGGGC
Amplicon Length (bp)	114
Chromosome Location	1:155260216-155260359
Assay Design	Exonic
Purification	Desalted

Validation Results

Efficiency (%)	102
R^2	0.9993
cDNA Cq	23.44

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cDNA Tm (Celsius)	84.5
gDNA Cq	24.19
Specificity (%)	100

Information to assist with data interpretation is provided at the end of this report.

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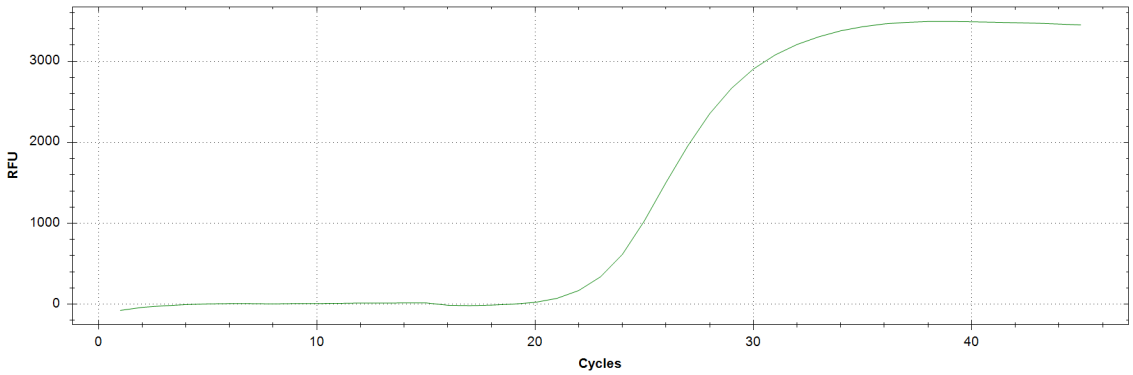
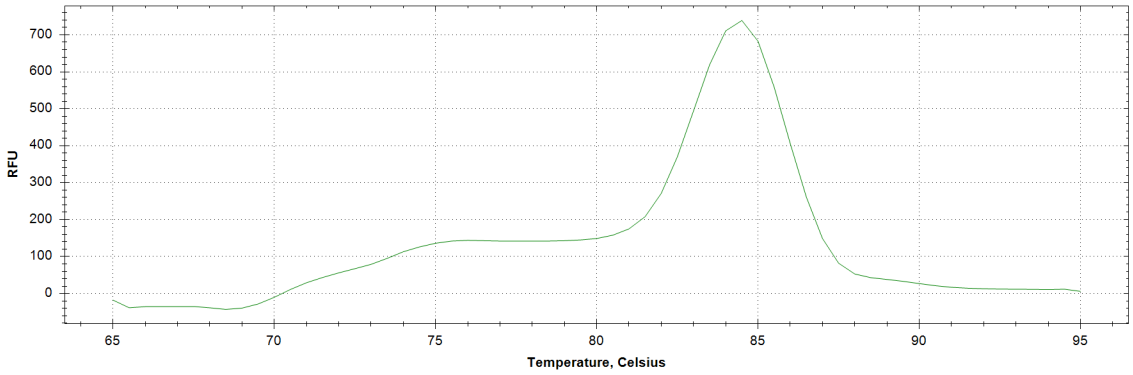
PKLR, Human

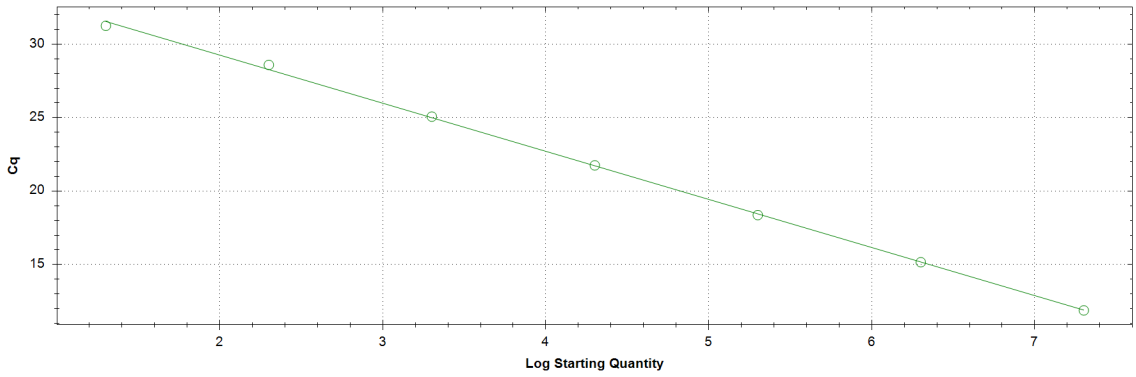
Amplification Plot
Amplification of cDNA generated from 25 ng of universal reference RNA



Melt Peak
Melt curve analysis of above amplification



Standard Curve
Standard curve generated using 20 million copies of template diluted 10-fold to 20 copies



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Products used to generate validation data

Real-Time PCR Instrument	CFX384 Real-Time PCR Detection System
Reverse Transcription Reagent	iScript™ Advanced cDNA Synthesis Kit for RT-qPCR
Real-Time PCR Supermix	SsoAdvanced™ SYBR® Green Supermix
Experimental Sample	qPCR Human Reference Total RNA

Data Interpretation

Unique Assay ID	This is a unique identifier that can be used to identify the assay in the literature and online.
Detected Coding Transcript(s)	This is a list of the Ensembl transcript ID(s) that this assay will detect. Details for each transcript can be found on the Ensembl website at www.ensembl.org.
Amplicon Context Sequence	This is the amplicon sequence with additional base pairs added to the beginning and/or end of the sequence. This is in accordance with the minimum information for the publication of real-time quantitative PCR experiments (MIQE) guidelines. For details, please refer to the following publication, "Primer Sequence Disclosure: A Clarification of the MIQE Guidelines" (Bustin et al 2011).
Chromosome Location	This is the chromosomal location of the amplicon context sequence within the genome.
Assay Design	Exonic: Primers sit within the same exon in the mRNA transcript and can potentially co-amplify genomic DNA. If performing gene expression analysis, it is suggested that the samples be treated with a DNase to eliminate potential unwanted signal from contaminating genomic DNA. Exon-exon junction: One primer sits on an exon-exon junction in mRNA. When performing gene expression analysis, this design approach will prevent unwanted signal from contaminating genomic DNA. Intron-spanning: Primers sit within different exons while spanning a large intron in the mRNA (intron is greater than 750bp). When performing gene expression analysis, this design approach should limit potential unwanted signal from contaminating genomic DNA. Small intron-spanning: Primers sit within different exons with a short intron in between (intron is smaller than 750bp). Small introns may not prevent unwanted signal from contaminating genomic DNA.
Efficiency	Assay efficiency was determined using a seven-point standard curve from 20 copies to 20 million copies. While an efficiency of 100% represents a perfect doubling of template at every cycle and is ideal, typical ranges of good assay efficiency are between 90-110%. For difficult targets, assay efficiency outside of this range are accepted and reported accordingly.
R^2	The R^2 represents the linearity of the standard curve and how well the standard curve data points fit the linear regression line. Acceptable values are >0.98.

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cDNA Cq	Cq value obtained from 25ng of cDNA transcribed from universal RNA when performing wet-lab validation of the assay. Note: Not all genes will be expressed at a detectable level in the universal RNA sample.
cDNA Tm	Melting temperature of the amplicon when running a melt curve analysis.
gDNA Cq	Cq value obtained when running the assay with 2.5ng of genomic DNA. This is more than a moderate level of genomic DNA contamination. Intron-spanning and exon-exon junction assay designs can minimize or eliminate genomic DNA detection. Note: Genomic DNA contamination is often present at variable levels. If concerned about genomic DNA contamination, the genomic DNA contamination control assay is recommended to run with your sample to determine if genomic DNA levels are sufficient to negatively impact results.
Specificity	This value is the percent of specific amplicon reads as measured by next generation sequencing (NGS). While 100% specificity is desirable, small decreases in specificity (<1%) can be due to NGS read errors. More significant reductions are likely due to co-amplification of homologous regions. Note: Since gene expression can be cell type and condition specific, the exact level and impact of co-amplification in a given sample is impossible to predict. If co-amplification is detected, it should be taken into consideration and reported when analyzing gene expression results.