

# PrimePCR™ Assay Validation Report

## Gene Information

Gene Name	breast cancer 1, early onset
Gene Symbol	BRCA1
Organism	Human
Gene Summary	This gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability and it also acts as a tumor suppressor. The encoded protein combines with other tumor suppressors DNA damage sensors and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex (BASC). This gene product associates with RNA polymerase II and through the C-terminal domain also interacts with histone deacetylase complexes. This protein thus plays a role in transcription DNA repair of double-stranded breaks and recombination. Mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers. Alternative splicing plays a role in modulating the subcellular localization and physiological function of this gene. Many alternatively spliced transcript variants some of which are disease-associated mutations have been described for this gene but the full-length natures of only some of these variants has been described. A related pseudogene which is also located on chromosome 17 has been identified.
Gene Aliases	BRCAI, BRCC1, BROVCA1, IRIS, PNCA4, PSCP, RNF53
RefSeq Accession No.	NC_000017.10, NG_005905.2, NT_010783.15
UniGene ID	Hs.194143
Ensembl Gene ID	ENSG00000012048
Entrez Gene ID	672

## Assay Information

Unique Assay ID	qHsaCED0038604
Assay Type	SYBR® Green
Detected Coding Transcript(s)	ENST00000357654, ENST00000468300, ENST00000493795, ENST00000471181, ENST00000491747, ENST00000484087, ENST00000478531, ENST00000493919, ENST00000487825, ENST00000470026, ENST00000477152, ENST00000494123, ENST00000473961, ENST00000476777, ENST00000354071, ENST00000352993, ENST00000346315, ENST00000351666, ENST00000393691, ENST00000309486
Amplicon Context Sequence	TTTGAGGTTGTATCCGCTGCTTGTCCCTCAGAGTTCTCACAGTTCCAAGGTTAGA GAGTTGGACACTGAGACTGGTTCCCTGCAAGGAAGGATTT
Amplicon Length (bp)	65
Chromosome Location	17:41251816-41256152
Assay Design	Exonic
Purification	Desalted

## Validation Results

Efficiency (%)	96
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R <sup>2</sup>	0.9995
cDNA Cq	21.97
cDNA Tm (Celsius)	79
gDNA Cq	24.45
Specificity (%)	100

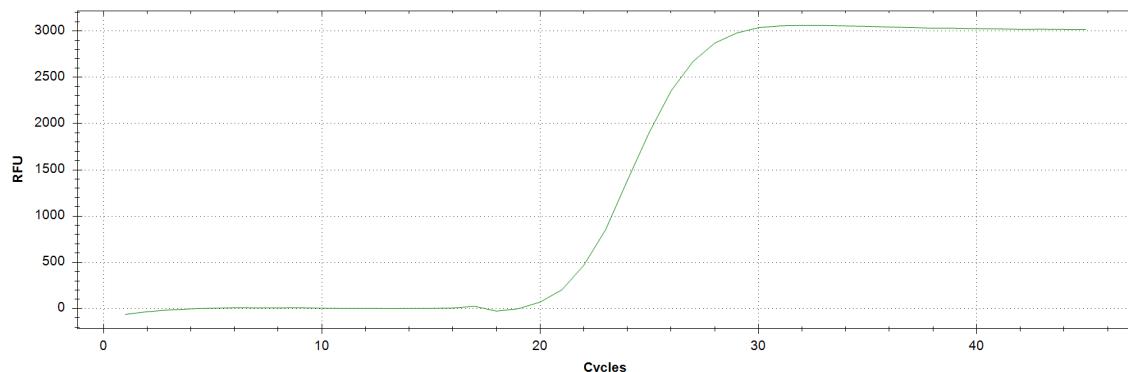
Information to assist with data interpretation is provided at the end of this report.

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BRCA1, Human

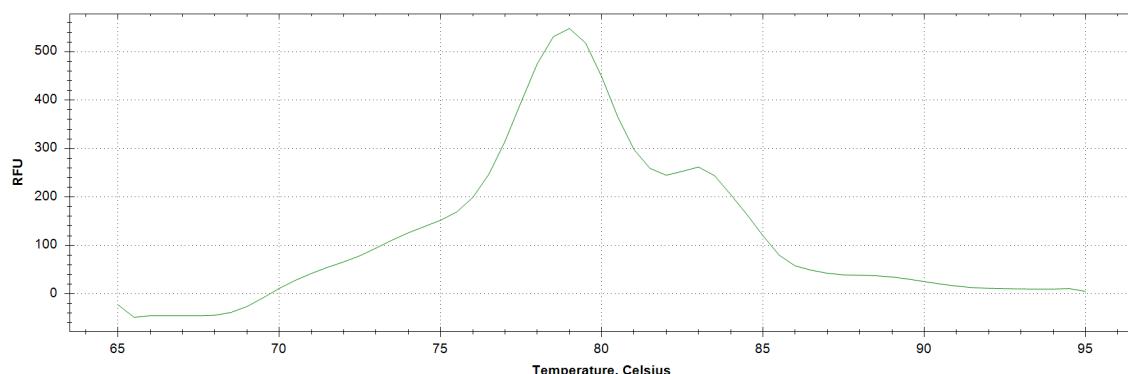
## Amplification Plot

Amplification of cDNA generated from 25 ng of universal reference RNA



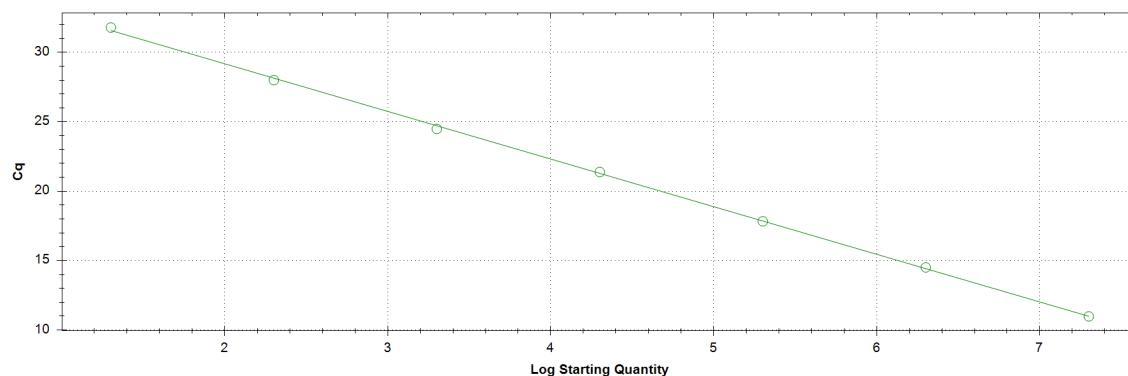
## Melt Peak

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

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

## Data Interpretation

<b>Unique Assay ID</b>	This is a unique identifier that can be used to identify the assay in the literature and online.
<b>Detected Coding Transcript(s)</b>	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 <a href="http://www.ensembl.org">www.ensembl.org</a> .
<b>Amplicon Context Sequence</b>	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).
<b>Chromosome Location</b>	This is the chromosomal location of the amplicon context sequence within the genome.
<b>Assay Design</b>	<p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p>
<b>Efficiency</b>	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.
<b>R<sup>2</sup></b>	The R <sup>2</sup> 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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<b>cDNA Cq</b>	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.
<b>cDNA Tm</b>	Melting temperature of the amplicon when running a melt curve analysis.
<b>gDNA Cq</b>	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.
<b>Specificity</b>	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.