PrimePCR™ Assay Validation Report

Gene Information

Gene Name	plectin isoform 1b
Gene Symbol	Plec
Organism	Rat
Gene Summary	Plectin is a prominent member of an important family of structurally and in part functionally related proteins, termed plakins or cytolinkers, that are capable of interlinking different elements of the cytoskeleton. Plakins, with their multi-domain structure and enormous size, not only play crucial roles in maintaining cell and tissue integrity and orchestrating dynamic changes in cytoarchitecture and cell shape, but also serve as scaffolding platforms for the assembly, positioning, and regulation of signaling complexes (for reviews see PMID: 9701547, 11854008, and 17499243). Plectin is expressed as several protein isoforms in a wide range of cell types and tissues from a single gene located on chromosome 8 in humans (PMID: 8633055, 8698233). Until 2010, this locus was named plectin 1 (symbol PLEC1 in human; Plec1 in mouse and rat) and the gene product had been referred to as "hemidesmosomal protein 1" or "plectin 1, intermediate filament binding 500kDa". These names were replaced by plectin. The plectin gene locus in mouse on chromosome 15 has been analyzed in detail (PMID: 10556294, 14559777), revealing a genomic exon-intron organization with well over 40 exons spanning over 62 kb and an unusual 5' transcript complexity of plectin isoforms. Eleven exons (1-1j) have been identified that alternatively splice directly into a common exon 2 which is the first exon to encode plectin's highly conserved actin binding domain (ABD). Three additional exons (-1, 0a, and 0) splice into an alternative first coding exon (1c), and two additional exons (2alpha and 3alpha) are optionally spliced within the exons encoding the acting binding domain (exons 2-8). Analysis of the human locus has identified eight of the eleven alternative 5' exons found in mouse and rat (PMID: 14672974); exons 1i, 1j and 1h have not been confirmed in human. Furthermore, isoforms lacking the central rod domain encoded by exon 31 have been detected in mouse (PMID:10556294), rat (PMID: 9177781), and human (PMID: 11441066, 10780662, 20052759). The short alternative amino-terminal sequences encoded by the different first exons direct the targeting of the various isoforms to distinct subcellular locations (PMID: 14559777). As the expression of specific plectin isoforms was found to be dependent on cell type (tissue) and stage of development (PMID: 10556294, 12542521, 17389230) it appears that each cell type (tissue) contains a unique set (proportion and composition) of plectin isoforms, as if custom-made for specific requirements of the particular cells. Concordantly, individual isoforms were found to carry out distinct and specific functions (PMID: 14559777, 12542521, 18541706). In 1996, a number of groups reported that patients suffering from epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) lacked plectin expression in skin and muscle tissues due to defects in the plectin gene (PMID: 8698233, 8941634, 8636409, 8894687, 8696340). Two other subtypes of plectin-related EBS have been described: EBS-pyloric atresia (PA) and EBS-Ogna. For reviews of plectin-related diseases see PMID: 15810881, 19945614. Mutations in the plectin gene related to human diseases should be named based on the position in NM_000445 (human variant 1, isoform 1c), unless the mutation is located within one of the other alternative first exons, in which case the position in the respective Reference Sequence should be used.
Gene Aliases	Not Available
RefSeq Accession No.	NM_001164296, NM_001164297, NM_001164298, NM_001164299, NM_001164302, NM_022401, NM_001164303, NM_001164304, NM_001164305, NM_001164307, NM_001164308

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UniGene ID	Rn.1085
Ensembl Gene ID	ENSRNOG00000023781
Entrez Gene ID	64204

Assay Information

Unique Assay ID	qRnoCEP0024557
Assay Type	Probe - Validation information is for the primer pair using SYBR® Green detection
Detected Coding Transcript(s)	ENSRNOT00000065445, ENSRNOT00000002083, ENSRNOT00000042642, ENSRNOT00000040762, ENSRNOT00000006311
Amplicon Context Sequence	CTGGTAGTGGCGACTACAAGATCCATATTCGCGCTCTGCCACCAGCCGGTCCTCGGGCCCAAAGCCACCAGCGTCCTGGCTGTCTCGAAGGAAGGCCTGGTAGTGCAACTCCAGGTTGCGCAGAGCTT
Amplicon Length (bp)	98
Chromosome Location	7:117230677-117230804
Assay Design	Exonic
Purification	Desalted

Validation Results

Efficiency (%)	101
R^2	0.9994
cDNA Cq	19.83
cDNA Tm (Celsius)	87.5
gDNA Cq	26.13
Specificity (%)	100

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

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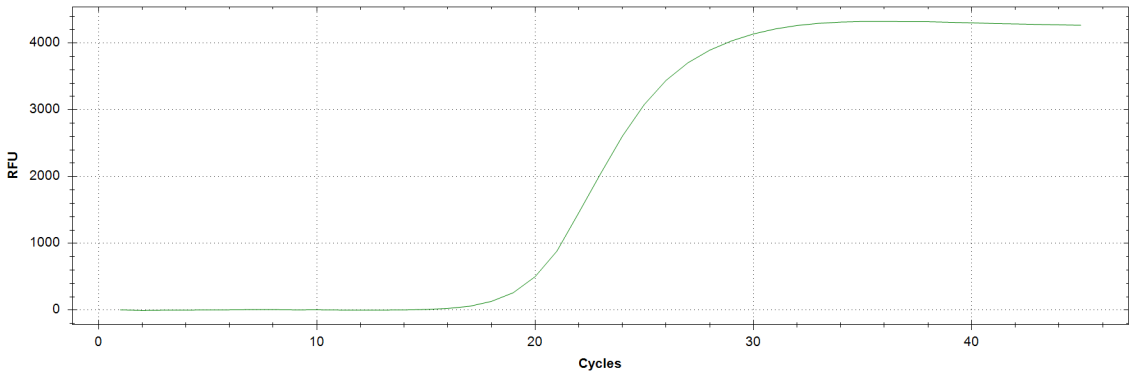
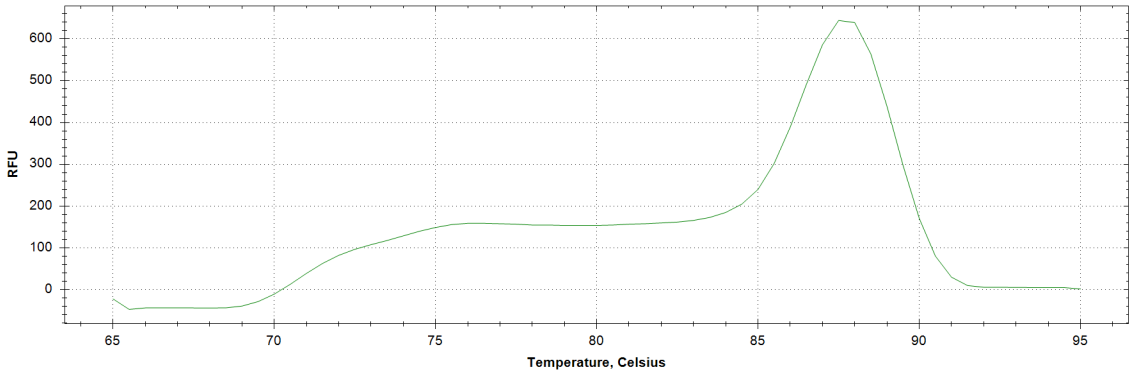
Plec, Rat

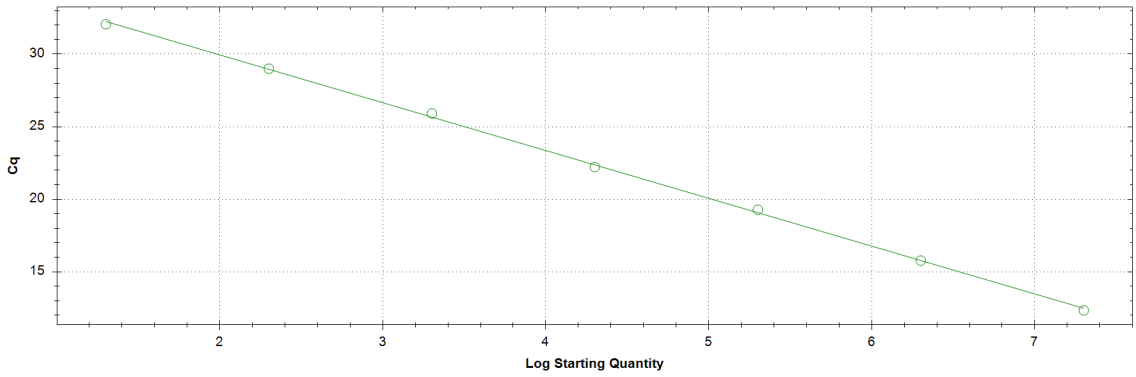
Amplification Plot
Amplification of cDNA generated from 25 ng of universal reference RNA



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 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.