

Catalog Number	Assay ID	RefSeq Accession Number	Gene Name	RT-qPCR product size (bp)	RT-qPCR Product T _m (°C)	PCR Product Location
95121-250	ACTB	NM_0011101	Actin, beta	71	84.5	Spans 1st intron in 5'UTR (exon 1 to exon 2)
95122-250	B2M	NM_004048	Beta-2-microglobin	95	79.5	Spans 2nd and 3rd introns (exon 2 to Exon 4)
95123-250	GAPDH	NM_002046	Glyceraldehyde-3-phosphate dehydrogenase	125	83.5	Spans 1st and 2nd introns (exon 1 to exon 3)
95124-250	GUSB	NM_000181	Glucuronidase, beta	80	80.0	Spans 8th intron (exon 8 to exon 9)
95125-250	HPRT1	NM_000194	Hypoxanthine phosphoribosyltransferase 1	107	80.0	Spans 6th intron (exon 6 to exon 7)
95126-250	PPIA	NM_021130	Peptidylprolyl isomerase A (cyclophilin A)	153	84.5	Spans 1st intron (exon 1 to exon 2)
95127-250	TFRC	NM_003234	Transferrin receptor (p90, CD71)	148	78.5	Spans 4th intron (exon 4 to exon 5) Amplifies both splice variants NM_001128148.1 and NM_003234.2"
95128-250	UBC	NM_021009	Ubiquitin C	63	76.5	Spans 1st intron (exon 1 to exon 2)
95129-250	YWHAZ	NM_003406	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	117	80.0	Spans 1st intron (exon 1 to exon 2)
95130-250	*RPL13A	NM_012423	Ribosomal protein L13a	134	80.5	* Exon 8, Does not differentiate mRNA/cDNA from gDNA

Description

Normalization of RT-qPCR data using one or more invariant reference genes is essential for accurate measurement of relative gene expression levels⁽¹⁻⁶⁾. PerfeCta Human Reference Gene Assays simplify reference gene selection by providing validated RT-qPCR performance when used with PerfeCta SYBR® Green SuperMix or PerfeCta SYBR Green FastMix®. Each assay delivers high qPCR efficiency (>95%), sensitivity and specificity. Primer sequences minimize or eliminate amplification of genomic DNA sequence (*when possible), exclude known single nucleotide polymorphisms (SNP), and minimize amplification of off-target homologous sequences. For additional information on available PerfeCta Reference Gene Assays, please visit our web site at www.quantabio.com.

Components

10 µM (each) mix of forward and reverse primer for indicated reference gene in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA.

Storage and Stability

PerfeCta Human Reference Gene Assays are stable for 3 years when stored in a constant temperature freezer at 20°C. For convenience, product may be stored unfrozen at +2 to +8°C for up to 6 months.

Guidelines for qPCR:

- Optimal primer concentration may vary between 100 and 300 nM depending on the choice of reaction plates and the PCR cycling program or thermalcycling properties of a given real-time PCR instrument. A final concentration of 200 nM for each primer is effective for most applications.
- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except sample template (cDNA) and dispense equal aliquots into each reaction tube. Always prepare a reaction cocktail volume that supports a greater number of reactions than the intended use so that there is sufficient volume for all reactions.
- Add DNA template to each reaction as the final step. Addition of samples as 2 to 5-µL volumes will improve assay precision. If necessary, dilute first-strand product in 10 mM Tris (pH8.0), 0.1 mM EDTA.
- After sealing each reaction, vortex gently to mix contents and then briefly centrifuge to collect components at the bottom of the reaction tube/well.

Reaction Assembly

Component	Volume for 20- μ L rxn.	Volume for 50- μ L rxn.	Final Concentration
PerfeCta SYBR Green SuperMix or FastMix (2X)	10.0 μ L	25.0 μ L	1x
PerfeCta Human Reference Gene Assay	0.4 μ L	1 μ L	200 nM each primer
Nuclease-free water	variable	variable	
1 st -strand cDNA from 1 to 100 ng of total RNA	variable	variable	
Final Volume (μ L)	20 μ L	50 μ L	

Note: For smaller or larger reaction volumes, scale all components proportionally.

PCR Cycling Protocol

	Recommended 2-Step Cycling Protocol	Optional 3-Step Cycling Protocol [§]
Initial Denaturation	95°C, 30 s	95°C, 30 s
PCR cycling (40 to 45 cycles)		
Denaturation	95°C, 5 to 10 s	95°C, 5 to 10 s
Annealing	60°C, 30 to 60s [†]	55 to 60°C, 20s
Extension		70°C [†] , 10s
Dissociation (melt curve) analysis	Consult instructions for your instrument	

* Full activation of AccuStart Taq DNA polymerase occurs within 30s at 95°C; however, optimal initial denaturation time is *template dependent* and will affect PCR efficiency and sensitivity. Amplification of genomic DNA or supercoiled DNA targets may require 5 to 10 min at 95°C to fully denature the template.

† Collect and analyze kinetic PCR data at the end of the extension step.

[§] **3-Step Cycling Protocol** can be used with PerfeCta Human Reference Gene Assays to provide compatibility with primer designs for other genes of interest (GOI). PerfeCta Human Reference Gene Assays support flexible cycling protocols while still providing highly specific and efficient amplification. Extension time may vary depending on the amplicon length for other GOIs as well as minimal data collection time requirement for your qPCR instrument.

Quality Control

Kit components are free of contaminating DNase and RNase. Reference gene assay sequences have been validated using PerfeCta SYBR Green SuperMix or PerfeCta SYBR Green FastMix for RT-qPCR of each mRNA in log-fold serial dilutions of cDNA product prepared using qScript cDNA SuperMix and reference RNA template. Linear regression analysis of the standard curve slope was between -3.20 and -3.35 with a R² > 0.995.

References

- 1) Livak KJ, and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25(4):402-8.
- 2) Pfaffl, MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* 29(9): 2001-2007.
- 3) Vandesompele, J. *et al.* (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple control genes. *Genome Biology* 3(7):research0034.1–0034.11
- 4) Hugget, J. *et al.* (2005) Real-time RT-PCR normalisation; strategies and considerations. *Genes Immun* 6, 279-284.
- 5) Vandesompele, J. *et al.* (2009) Reference Gene Validation Software for Improved Normalization. in *Real-Time PCR: Current Technology and Applications*, Publisher: Caister Academic Press. Editor: Julie Logan, Kirstin Edwards and Nick Saunders ISBN: 978-1-904455-39-4
- 6) Derveaux, S. *et al.* (2010) How to do successful gene expression analysis using real-time PCR. *Methods* 50 (2010) 227–230