

User Manual



Reference Gene Panel  
Human  
Probe protocol

*Version 1.1 — August 2014*

*For use in quantitative real-time PCR*



tataabiocenter



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## Background

For accurate gene expression measurements it is necessary to normalize results of the expression of target genes to a reference, which is not affected by the parameters studied in the specific study. There exists to our knowledge no reference gene that is unaffected in all conditions. Therefore it is necessary to find the optimal reference gene for your conditions and validate that this gene is indeed non-regulated.

For this reason we have developed the Human Reference Gene Panel which contains primer- and probe sets for 12 potential reference genes in human. The expression of these genes should be measured on a number of representative samples, and from the results the most constantly expressed gene(s) can be determined. These genes are then used for subsequent normalization of target gene expression for more accurate results.

## Assays included in the panel

The primers are designed to span exon-boundaries. Adequate efficiency of amplification was determined for each assay on commercial kits using FAM & BHQ1.

Gene	Full name	Expression level	PCR product size
ACTB	Actin, beta	High	188 bp
B2M*	Beta-2-microglobulin	Medium	161 bp
GAPDH**	Glyceraldehyde-3-phosphate dehydrogenase	High	151 bp
GUSB	Beta-glucuronidase	Low	165 bp
HPRT1	Hypoxanthine-guanine phosphoribosyltransferase	Low	94 bp
PPIA	Peptidylpropyl isomerase A, Cyclophilin A	Low	114 bp
RPLP	60S acidic ribosomal protein P0	Medium	150 bp
RRN18S*	18S rRNA	Very High	120 bp
TBP	TATAA-box Binding Protein	Medium	174 bp
TUBB	Tubulin, beta polypeptide	High	119 bp
UBC	Ubiquitin C	Low	240 bp
YWHAZ	Tyrosine 3/tryptophan 5 -monooxygenase activation protein, zeta polypeptide	Low	248 bp

\*The B2M and 18S rRNA assays are designed within an exon and may amplify genomic DNA.

## Contents

- Positive Control DNA
- Primer solutions for 12 potential human reference genes  
100 rxn (100  $\mu$ l of primer mix, C=10  $\mu$ M per primer)
- Probe solutions for 12 potential human reference genes  
100 rxn (50  $\mu$ l of probe mix, C=10  $\mu$ M per probe)
- GenEx Standard software, 1 year license (only for first time users, see p. 7)

## Additionally required materials and devices

### • **Real-time PCR instrumentation**

This kit has been validated on several different instrument platforms. It is suitable for use on all real-time PCR instruments.

### • **Master mix or master mix components**

This kit has been validated using a variety of commercially available master mixes with good results. For best results in probe based qPCR TATAA Biocenter recommends to use the TATAA Probe GrandMaster® Mix.

### • **Pipettes and tips**

### • **Vortex and centrifuge**

### • **Sample cDNA**

It is recommended to use cDNA of high quality. RNA integrity should be assessed prior to cDNA synthesis e.g. using Advanced Analytical Fragment Analyzer™ Automated CE System, Agilent 2100 Bioanalyzer, Agilent ScreenTape® system or BioRad Experion.

## Storage

The contents of this kit can be stored 1 month at +4°C. For long term storage -20°C is recommended. Repeated freeze-thaw cycles should be avoided. Vortex thoroughly and spin down before use.

## Amplification protocol

The amplification protocol below is recommended. Optionally a slow mastermix may be used, then we recommend 95°C 15s and 60°C 45s, repeated 40 cycles.

UNG step (optional)	according to mix instructions		
Enzyme activation	according to mix instructions		
Cycling (x40)	denaturation	5s	95°C
	annealing & elongation	30s	60°C

## Pipetting protocol

We recommend that each sample is quantified in duplicate or triplicate for each gene in the panel. Approximately 5-10 samples of each type (healthy/sick, treated/non-treated etc.) should be evaluated for determination of the most constantly expressed gene. Equal numbers of samples from each sample type should be included in the test.

Prepare master mixes for each gene according to the protocol to the right. Prepare at least 1 reaction extra so that you do not run out of master mix during the pipetting.

Component	1 rxn	1 rxn	1 rxn
PCR-Grade water	3.4µl	6.8µl	9µl
Primer mix	0.4µl	0.8µl	1µl
Probe mix	0.2µl	0.4µl	0.5µl
Master mix (2X)	5µl	10µl	12.5µl
cDNA	1µl	2µl	2µl
Final Volume	10µl	20µl	25µl

Larger volume of cDNA may be added. In this case change the volume of water accordingly. 2µl of Positive Control DNA can be added as template as an additional control of the PCR reaction. A negative control with water instead of cDNA should always be included as a control of contamination of the reagents.



GenEx Standard is included as a time limited license when purchasing the Reference Gene Panel. The GenEx software is also available in Professional and Enterprise versions. Besides the functionality in GenEx Standard, GenEx Professional also includes Self Organizing Maps, Principle Component Analysis, and basic statistical analysis. GenEx Enterprise also includes advanced Neural Network modules and Experimental Design Optimization.

	GenEx Standard	GenEx Pro	GenEx Enterprise
User friendly data pre-processing	X	X	X
GeNorm	X	X	X
Normfinder	X	X	X
Scatter plots and 3D visualization	X	X	X
Hierarchical Clustering	X	X	X
Correlation analyses	X	X	X
Self Organizing Maps		X	X
Heatmap Analysis		X	X
Histogram		X	X
Principle Component Analysis		X	X
CLSI EP6-Linearity test			X
Trilinear Decomposition			X
Experimental Design Optimization			X
Artificial Neural Networks			X

To purchase a non-time limited license, to upgrade your GenEx to a more advanced version, or for more information about versions and licenses please contact [order@tataa.com](mailto:order@tataa.com).

## Reorder Information

The Human Reference Gene Panel Probe has product number **A101P**. When the optimal reference genes for your study has been determined, those genes can be ordered individually (product no [qA-01-0101P](#) to [qA-01-0112P](#)) from our webshop.



## Troubleshooting

### • **I do not get any amplification/signal?**

The instrument may not have been programmed correctly or there may be a problem with your master mix. Evaluate if the problem is in the detection or the amplification by running the PCR-product on a gel. Run a new test using the positive control DNA provided with the kit.

### • **My negative controls are amplified?**

This indicates that your final master mix has been contaminated. Run a new test using the positive control DNA provided with the kit and a different water source.

### • **My samples have same/higher Cq-value than my no template controls?**

This indicates that you have added too little cDNA. Add more cDNA and try again. The cDNA may be of low quality. Check the quality of the RNA before performing cDNA synthesis. If your no template controls are positive, this indicates contamination of the water or the master mix.

### • **My replicates are not very tight?**

With good quality cDNA and good pipetting technique, very high reproducibility is expected. Low amounts of cDNA can lead to higher variation. Also, low quality cDNA can lead to differences between replicates. Check the accuracy and reproducibility of your pipettes.

### • **The signal for 18S rRNA amplifies very early and does not have a good baseline?**

In some cases the amount of 18S is too high which can result in erroneous background subtraction. Try diluting the cDNA sample.

### • **I get a positive amplification from genomic DNA**

Where possible, the assays in the Reference Gene Panel have been designed to span exon-boundaries. However, when intron-less pseudogenes are present in the genome, genomic DNA may still give a positive amplification. Try removing DNA contamination by DNase treating the RNA sample.

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## References

Vandesompele J., *et al.*, (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, 3(7) 0034.I - 0034.II.

Szabo A. *et al.*, (2004) Statistical modeling for selecting housekeeper genes. *Genome Biology*, 5:R59.

Andersen C.L., *et al.*, (2004) Normalization of Real-Time Quantitative Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data Sets. *Cancer Research*, 64, 5245–5250.

Stålberg A. *et al.*, (2005) Quantitative real-time PCR for cancer detection: the lymphoma case. *Exp. Rev. Mol. Diagn.* 5(2).

## Contact

For more information about the product and reference genes please contact us at [info@tataa.com](mailto:info@tataa.com).

## License information

PCR is covered by several patents owned by Hoffman-La Roche Inc. and Hoffman-LaRoche, Ltd. Purchase of the Reference Gene Panel Human does not include or provide a license with respect to any PCR-related patent owned by Hoffman-La Roche or others. TATAA Biocenter does not encourage or support the unauthorised or unlicensed use of the PCR process.

## qPCR training courses at TATAA Biocenter

TATAA Biocenter is a leading organizer of hands-on training in qPCR and related technologies. For comprehensive training program please visit [www.tataa.com](http://www.tataa.com).

## Other products from TATAA

### **ValidPrime™ - mouse, human and other vertebrates**

ValidPrime™ is an assay to test for the presence of gDNA in test samples and when combined with a gDNA control sample, replaces all RT(-) controls. ValidPrime™ is highly optimized and specific to a non-transcribed locus of gDNA that is present in exactly one copy per haploid normal genome. The kit also contains a gDNA standard that can be used to test the sensitivity of RT-qPCR assays for gDNA background. ValidPrime™ replaces the need to perform RT(-) controls for all reactions and makes RT-qPCR profiling easier and substantially cheaper.

### **(HL-)dsDNase**

New generation DNase that is specific to double stranded DNA and can be efficiently inactivated by heating at 55 °C. It can be added to your RT reaction to efficiently remove any gDNA, without degrading single-stranded cDNA. It is completely inactivated by the PCR and does not degrade the double stranded PCR product.

### **GenEx software**

GenEx provides the appropriate tools to analyze real-time PCR gene expression data and to extract valuable information from the measurements.

### **TATAA Interplate Calibrator - Variation Compensation**

For practical reasons many qPCR studies involve the use of samples that are processed in more than a single batch or in which the sample set is extended over time. Even over a short time period, variation between qPCR processing runs is observed due to different baseline subtractions and threshold settings. The TATAA Interplate Calibrator (IPC) is used to compensate for the variation between qPCR runs.

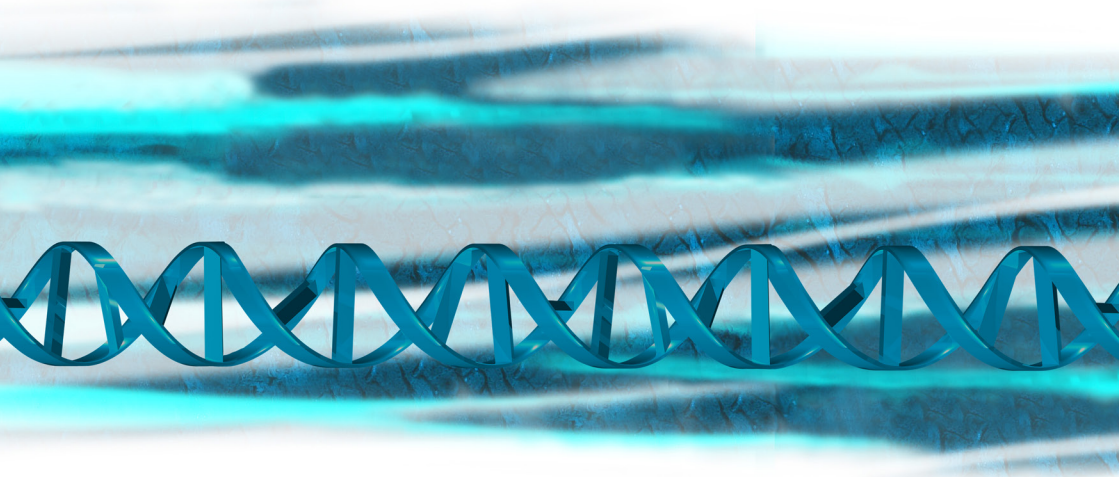
### **CelluLyser™ - for rapid and easy lysis and cDNA synthesis**

The CelluLyser™ Lysis and cDNA Synthesis Kit enables you to generate cDNA from small samples with minimal losses and hands-on time. It is particularly useful for single cell analysis. By using CelluLyser™, the entire workflow from cell lysis to RT and qPCR can be performed without washing steps, thus eliminating material loss.

### **VisiBlue™ mastermix colorant**

The VisiBlue™ mastermix colorant enables you to quickly color your favourite qPCR mastermix to easily visualize where the reagent has been added to your plates and tubes. VisiBlue™ is very easy to use by a simple addition to your favorite master mix.

# Express your genius



TATAA Biocenter, with offices in Gothenburg, Saarbrücken and Prague, is the leading provider of real-time PCR services and the prime organizer of real-time PCR workshops globally. TATAA Biocenter conducts commissioned research and training within the

field of molecular diagnostics and gene expression analysis, along with developing real-time PCR expression panels. TATAA Biocenter has great experience and expertise in high resolution gene expression profiling, pathogen detection, and small sample/single cell analysis.



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