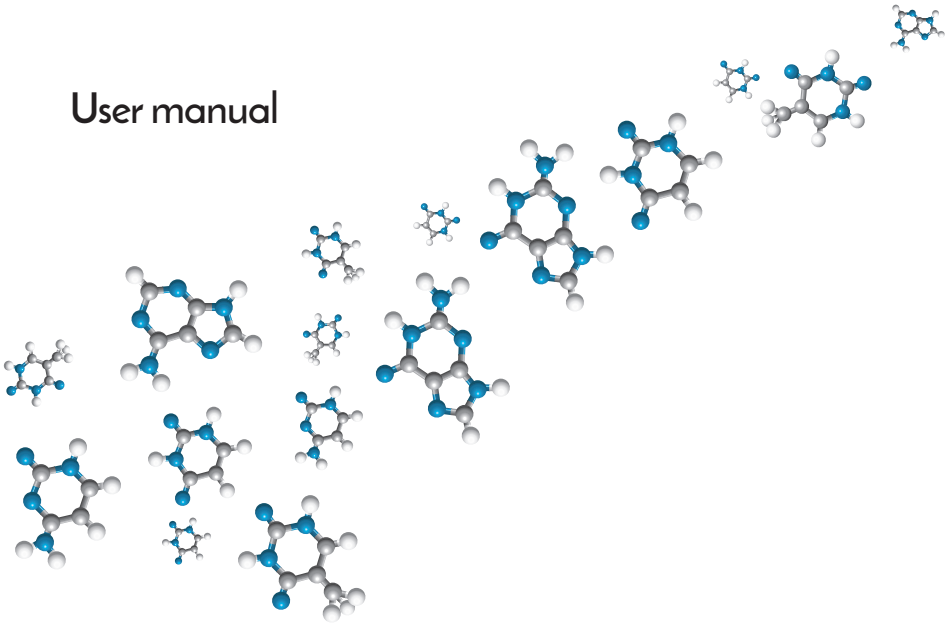
User manual



VisiBlue™
qPCR master mix colorant

Version 1.3 - February 2018

Order #:

2000 rxn: K101a

tataabiocenter

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Background

VisiBlue™ is an inert coloring dye developed by TATAA Biocenter for use in quantitative real-time PCR (qPCR) applications. The dye can be added to a PCR mix and does not interfere with the PCR amplification or the detection of product using dsDNA binding dyes or labelled probes. Coloring the reagents enables easy handling and easier detection of pipetting errors.

VisiBlue™ can be used as an inert colorant for qPCR applications or other applications where staining of reagents are wanted.

Contents

500 µl of 80x stock solution of VisiBlue™. Sufficient for approximately 2000 PCR reactions of 20 µl.

Absorbance and fluorescence

VisiBlue™ has an absorbance maximum at 619 nm. Tests show that VisiBlue™ does not significantly interfere with the emission of SYBR Green, FAM, JOE, Texas Red and ROX.

Storage

The stock solution is supplied in TE-buffer and is stable at +4°C. Once added to PCR mix the dye can be stored at +4°C for at least 1 month. Repeated freeze-thaw cycles are not recommended.

Additionally required materials and devices

- **Real-time PCR instrumentation:** The The product has been validated on several different instrument platforms.
- **Master mix:** VisiBlue™ has been validated in a large number of master mixes using conditions recommended by the manufacturers.

Note: The use of VisiBlue™ in certain mixes results in a slightly lower fluorescence signal.

- **Pipettes and tips**
- **Vortex and centrifuge.**
- **Experimental sample (DNA/cDNA).** Optimal results in qPCR require high quality DNA or cDNA samples. Quality of RNA can be tested prior to cDNA synthesis using Agilent 2100 Bioanalyzer or Bio-Rad Experion.

Typical results using VisiBlue™

VisBlue™ can be used as an inert reagent colorant for qPCR applications.

The end fluorescence level when adding VisiBlue™ is only slightly reduced. This reduction varies with the mix that is used. The melting temperature of the PCR product is generally not affected, so optimization of previously well functioning assays should not be necessary when using VisBlue.

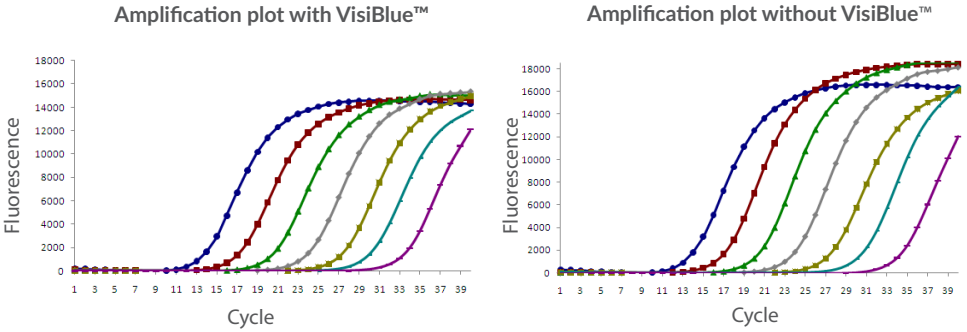


Figure 1. Amplification is not affected by the addition of VisiBlue™. 18S rRNA was amplified from control DNA samples. VisiBlue™ was added directly to the Finnzymes DyNAmo qPCR mastermix and shows no significant difference in end fluorescence compared to the master mix without VisiBlue™.

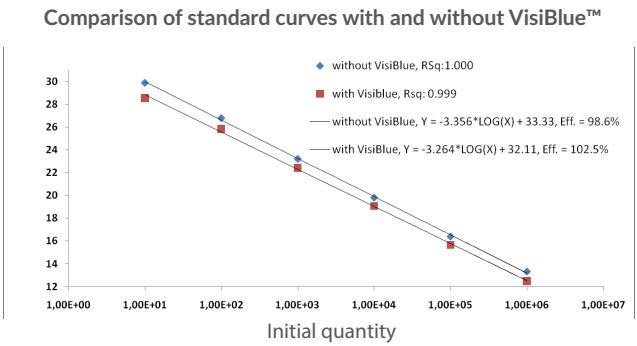


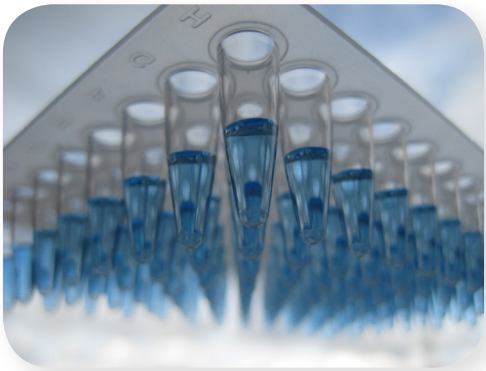
Figure 2. No influence of addition of VisiBlue™ on qPCR performance. Comparing standard curves with and without the addition of VisiBlue™ indicates that no inhibition takes place. Slight shift in Cq is observed because of different threshold values.

Instructions for use

Add 25 µl of VisiBlue™ to 1 ml of a 2x mix. The increase in volume is negligible. If larger or smaller volumes of mix are desired to be colored, simply scale the amount of VisiBlue™ added proportionally.

Safety

Regular precautions should be taken when using and handling VisiBlue™. Do not inhale/ consume or let in contact with skin or eyes.



Trouble shooting

• I do not get any amplification/signal?

The instrument may not have been programmed correctly. Make sure that you are monitoring the appropriate channel and that the instrument is calibrated if necessary. If you run the samples on a gel and get visible bands you know that the template has been amplified and the problem lies in the detection or vice versa. VisiBlue has not been shown to affect amplification but may in some instances reduce fluorescence signal.

• I get a very noisy amplification and dissociation curve?

The quality of the signal may depend on the chemistry used and on the filter settings on the qPCR platform. If the instrument used has variable gain setting, try to increase the gain. If the instrument requires calibration, make sure that this has been performed. Changing the master mix can also improve the signal.

• My replicates are not tight?

With good quality DNA and good pipetting technique, very high reproducibility is possible. Low amounts of DNA can lead to higher variation. Also, low quality DNA can lead to differences between replicates.

• My negative controls are amplified?

Your reagents are probably contaminated. If using a dsDNA binding dye, keep in mind that the dye will also bind to non-specific products such as primer dimers. Amplification of non-specific products will lead to an amplification signal. The amplified product can be evaluated by doing dissociation curve analysis.

• My samples have same/higher Cq-value than my NTC?

This indicates that you have added too little DNA. Add more DNA and try again. The DNA may be of low quality. Check the quality of the RNA before doing cDNA synthesis. If you are using a probe, this indicates that you have a contamination..

License information

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

Contact and ordering information

To re-order the VisiBlue™or for more information about the product and other products available from TATAA Biocenter, please contact us on order@tataa.com, visit our website www.tataa.com or contact your local TATAA distributor in your country.

Other products from TATAA

Universal RNA/DNA Spike - tests for inhibition and yield

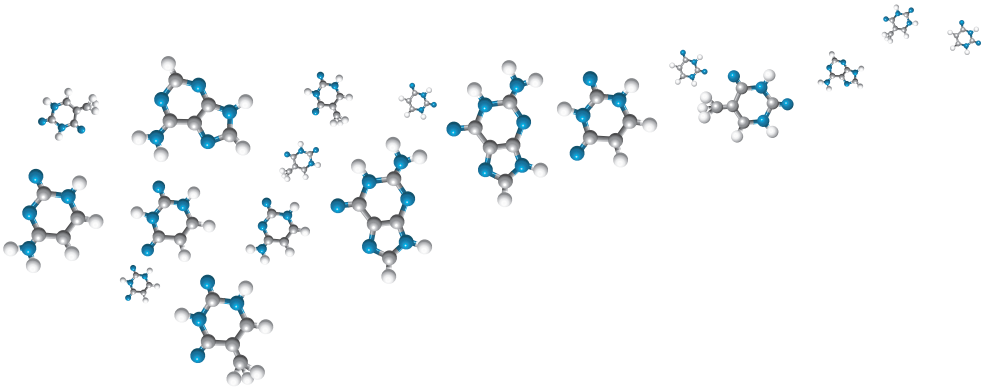
The TATAA Universal Spike is an easy to use and very effective tool for quality control throughout the entire RT-qPCR experimental workflow. The Cq of the Spike assay also reflects losses during extraction, handling, transport and storage of samples, including freeze-thaw events during RT-qPCR.

Reference Gene Panel - Human, Mouse or Rat

The panel contains primer sets for 12 commonly used reference genes. A perfect product for finding the most optimal reference gene for your samples.

ValidPrime®- genomic DNA control

ValidPrime® is an assay to test for the presence of gDNA in samples and when combined with a gDNA control sample, replaces all RT(-) controls. ValidPrime® is highly optimised 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.



Express your genius

TATAA Biocenter, with offices in Gothenburg, Sweden, and Prague, Czech Republic, is the leading provider of quantitative real-time PCR and NGS services and the prime organiser of quantitative real-time PCR and NGS 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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