

User Manual



VisiBlue™
qPCR mix colorant

Version 1.2 — March 2010

For use in quantitative real-time PCR



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Background

VisiBlue™ is an inert coloring dye developed by TATAA Biocenter for use in among other things real-time PCR 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 real-time PCR applications or other applications where staining of reagents are wanted.

Contents

480 µl of 83.5x stock solution of VisiBlue™. Sufficient for approximately 2000 PCR reactions of 20 µl.

Absorbance and fluorescence

VisiBlue™ has an absorbance maximum at 616 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 can be stored at +4°C for up to 12 months. 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 product has been validated on several different instrument platforms.

- Mastermix or mastermix components

VisiBlue™ has been tested using many commercially available mixes, including Quantace SensiMix, Roche LightCycler® 480 Probes Master, Fermentas Maxima™ Probe qPCR mastermix, Finnzymes DyNAmo™ Flash Probe kit, Quantace SensiMix HRM™ and PrimerDesign Precision™ HRM MasterMix.

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

- Pipettes and tips

- Vortex and centrifuge

- Sample cDNA

Optimal results in real-time PCR require high quality DNA samples. Quality of RNA can be tested prior to cDNA synthesis using Agilent 2100 Bioanalyzer or Bio-Rad Experion.

Typical results using VisiBlue™

VisiBlue™ can be used as an inert reagent colorant for real-time PCR 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 VisiBlue.

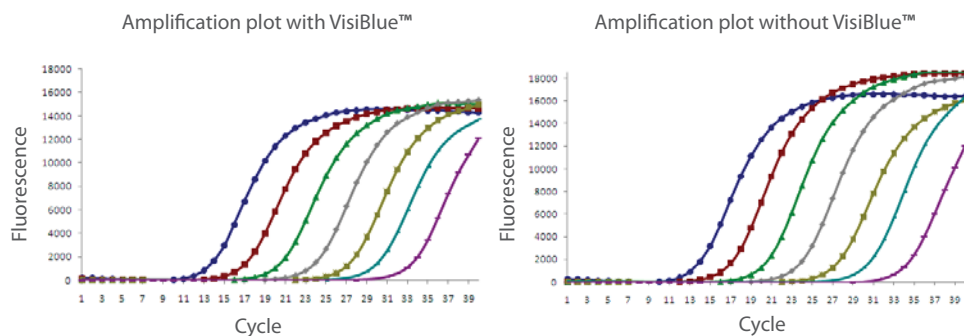


Figure 1. Amplification is not affected by the addition of VisiBlue™. 18S rRNA was amplified from a control DNA samples. VisiBlue™ was added directly to the Finnzymes DyNAmo qPCR mastermix and shows no significant difference in end fluorescence compared to the mastermix without VisiBlue™.

Instructions for use

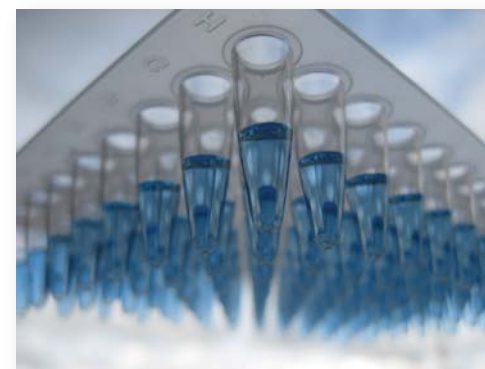
480 µl of a 83.5x stock solution is supplied in TE-buffer and is stable for up to 12 months at +4°C.

To use add 24 µl of VisiBlue™ to 1 ml of a 2x mix. The increase in volume is neglectable and this mix can still be considered 2x. If larger or smaller volumes of mix are desired to be colored, simply scale the amount of VisiBlue™ added proportionally.

VisiBlue™ is stable for at least 1 month at +4°C once added to the mix.

Safety

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



Comparison of standard curves with and without VisiBlue™

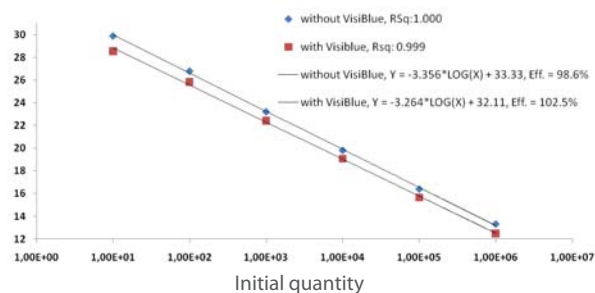


Figure 2. No influence of addition of VisiBlue™ on qPCR performance. Comparing standard curves with and without the addition of VisiBlue™ indicates that no inhibition takes place. Slight shift in Cq is observed because of different threshold values.

Troubleshooting

• 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 signal may depend on the chemistry used and on the filter settings on the real-time PCR platform. If the instrument used has variable gain setting, try to increase the gain. Also make sure that if the instrument requires calibration that this has been performed. Changing the mastermix can also improve the signal.

• My negative controls give a positive amplification?

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 Ct-value than my negative controls?

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.

• My replicates are not very 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 big differences between replicates.

Contact

For more information about the product and other products available from TATAA Biocenter please contact us on info@tataa.com or visit our website www.tataa.com.

License information

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for commercial purposes. For information on purchasing a license to this product for purposes other than research, contact TATAA Biocenter AB, Odinsgatan 28, S-41103 Göteborg, Sweden, Phone: +46 31 761 57 00, Fax: +46 31 152890, Email: info@tataa.com

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Other products from TATAA

Human Endogenous Control Gene Panel

A panel containing primer sets for 12 commonly used human reference genes. A perfect product for finding the most optimal reference gene for your samples. GenEx Standard software is also included in the kit.

Mouse Endogenous Control Gene Panel

A panel containing primer sets for 12 commonly used mouse reference genes. A perfect product for finding the most optimal reference gene for your samples. GenEx Standard software is also included in the kit.

Chromofy A dye for qPCR and HRM

Chromofy is a monomeric asymmetric cyanine dye, developed for use in qPCR applications. The dye has absorbance and emission wavelengths that are suitable for the FAM/SYBR channel on most common real-time PCR instruments.

GenEx software

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

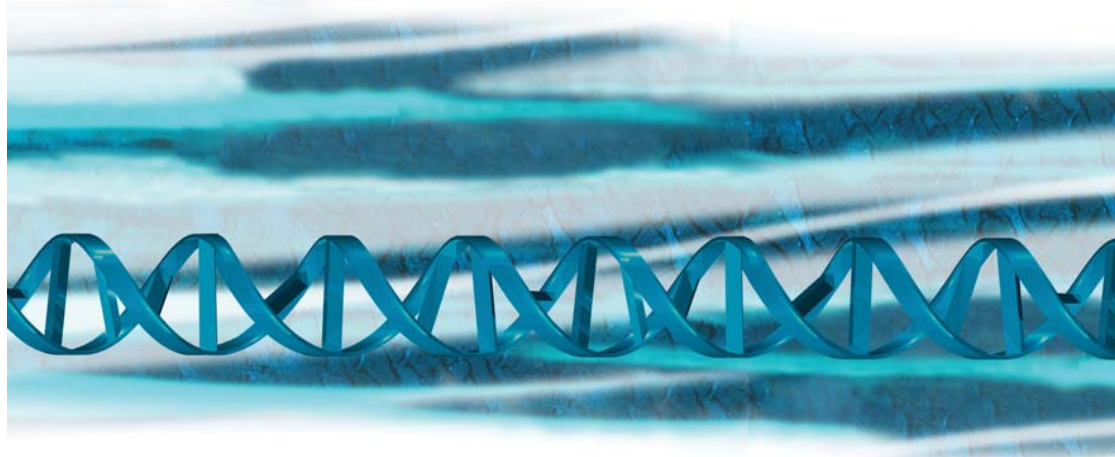
TrulyStem - for Human Embryonic Stem Cell Characterization

For easy monitoring of the differentiation state of stem cells using real-time PCR. The kit contains primer sets for 13 well established stem cell differentiation markers and positive control cDNA from differentiated and non-differentiated stem cells.

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, allowing even a single cell to be analysed. A rapid and sensitive lysis is followed by reverse transcription without the need of purification.

Express your genius



TATAA Biocenter, with offices in Gothenburg, San Francisco, 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 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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