User Manual

BOXTOM
Control for Primer Dimers in Probe based qPCR assays

Version 1.8 — Oct 2012
For use in quantitative real-time PCR

tataabiocenter
Table of contents

Background .................................................................................................................. 4
Fluorescence data ......................................................................................................... 4
Best of both worlds ....................................................................................................... 5
Contents ....................................................................................................................... 6
Additionally required materials and devices ................................................................. 6
Storage ........................................................................................................................ 7
Instructions for use ....................................................................................................... 7
Troubleshooting ........................................................................................................... 8
References ................................................................................................................... 9
Contact ........................................................................................................................ 9
License information .................................................................................................... 9
Other products from TATAA ..................................................................................... 10

ValidPrime™ Control for Genomic Background
GenEx software
Reference Gene Panel - Human or Mouse
VisiBlue qPCR mix colorant
CelluLyser™ - for rapid and easy lysis and cDNA synthesis
TrulyStem™ - for Human Embryonic Stem Cell Characterization
Background

BOXTO is a derivative of BEBO, an unsymmetric cyanine dye developed by TATAA Biocenter for use in quantitative real-time PCR (qPCR) applications. The dye has absorbance and emission wavelengths that can be detected on the JOE channel on most common qPCR instrumentation and shows a very strong fluorescence increase when bound to dsDNA.

BOXTO can be used as an unspecific dye for qPCR applications or other applications where staining of dsDNA is wanted. The BEBO-family of dyes are patent pending.

Fluorescence data

Maximum absorbance at 515 nm and maximum emission at 552 nm, when bound to dsDNA. Fluorescence increase is more than 250 times when bound to dsDNA. Maximum absorbance free in solution is at 482nm.
Best of both worlds

Since unspecific dyes also detect artefacts such as primer dimers, in some cases using sequence specific probes may be beneficial. On the other hand, when using probes dissociation curves cannot be performed to study what has been amplified in each reaction, and in some cases gel electrophoresis has to be performed to troubleshoot, leading to extremely high risk of contamination.

BOXTO can be used in combination with any FAM-labeled probe and a dissociation curve can be performed after amplification. Quantification is then performed on the FAM-channel and dissociation curve on the JOE-channel.

**Figure:** Using BOXTO in combination with a molecular beacon FAM-labeled probe. Acquiring on the FAM-channel does not detect the NTC containing only primer dimers (red curves). These are detected in the JOE-channel with BOXTO (top right) in the same reaction tube. Dissociation curve analysis can be performed after amplification using BOXTO on the JOE-channel. Here 0.7 µM of BOXTO was used in combination with 0.4 µM of the molecular beacon probe.
Contents

• 5mM stock solution of BOXTO in DMSO. Sufficient for approximately 6000 rxns.

Additionally required materials and devices

• **Real-time PCR instrumentation**
  This kit has been validated on several different instrument platforms.

  *Note: Using BOXTO on certain instruments may need calibration. Most instruments can directly use the JOE-channel to monitor the BOXTO signal.*

• **Mastermix or mastermix components**
  The dye has been tested using many commercially available mastermixes, including Eppendorf Realplex Mastermix, Invitrogen Platinum qPCR Supermix-UDG, BioRad iQ Supermix, Quantace Sensimix, KAPA probe FAST Universal mix.

  *Note: The use of BOXTO in certain mastermixes results in a lower and noisier amplification and dissociation curve signal. This has been noticed for example with Applied Biosystems TaqMan Universal Mastermix.*

  **BOXTO is compatible with ROX normalisation and dUTP containing mastermixes.**

• **Pipettes and tips**

• **Vortex and centrifuged**

• **Sample cDNA**
  When using cDNA as sample it is recommended to use cDNA of high quality. Quality of RNA can be checked prior to cDNA synthesis using Agilent 2100 Bioanalyzer or BioRad Experion.
Storage
The stock solution can be stored at -20°C for 12 months. Once diluted in water the dye is preferably stored at +4°C for 1 month. Repeated freeze-thaw cycles are not recommended.

Instructions for use
15 µl of stock solution is supplied at 5 mM in DMSO and is stable for 12 months in -20°C.

Before use dilute the stock to a working solution of 50 µM in mQ water by diluting the stock 1 µl + 99 µl. A solution of 50 µM (a 1+99 dilution) is stable for more than 1 month at 4°C. Typical concentration in real-time PCR reactions is approximately 0.5 µM (concentrations around 0.3-2 µM can be tested). Detection should be done on the JOE channel. The dye can be used with most commercially available real-time PCR reagents. 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 settings, BOXT0 should generally be used at a high gain setting.
Troubleshooting

• I do not get any amplification/signal?
The instrument may not have been programmed correctly. You can evaluate if the problem is in the detection or the amplification by running the samples on gel. Make sure the BOXTO dye is monitored in the appropriate channel and that the instrument is calibrated if necessary.

• 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 qPCR platform. If the instrument used has variable gain setting, try to increase the gain. If the instrument requires calibration make sure also that this is performed. Changing the mastermix can also improve the signal.

• My negative controls give a positive amplification?
Since BOXTO is a dsDNA binding dye it 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 negative controls?
This indicates that you have added too little DNA. Add more DNA and try again. The DNA may also be of low quality. Check the quality of the RNA before doing cDNA synthesis.

• 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.
References


Contact

For more information about the product and other products available from TATAA Biocenter contact us on support@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, Medicinaregatan 8A, S-41346 Göteborg, Sweden, Phone: +46 31 741 18 00, Fax: +46 31 741 17 01, Email: info@tataa.com

PCR is covered by several patents owned by Hoffman-La Roche Inc. and Hoffman-LaRoche, Ltd. Purchase of the product 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.
**Other products from TATAA**

**ValidPrime™ Control for Genomic Background**
ValidPrime™ replaces the need to perform no reverse transcriptase (RT(-)) controls for all samples in your qPCR profiling to test for the presence of genomic DNA (gDNA). Just add the ValidPrime™ assay to the list of assays, and the gDNA control to the list of samples, and run. ValidPrime™ will minimize the amount of control reactions and hence your costs, as well as your efforts.

**GenEx software**
Market leading software for qPCR analysis. GenEx provides the appropriate tools to analyze qPCR gene expression data and to extract biologically relevant information from the measurements.

**Reference Gene Panel - Human or Mouse**
The panel contains primer sets for 12 commonly used human or mouse reference genes. A perfect product for finding the most optimal reference gene for your samples. GenEx Standard software with GeNorm and Normfinder is also included in the kit.

**VisiBlue qPCR mix colorant**
The VisiBlue mastermix colorant enables you to color your favourite qPCR mastermix to easily visualize where the reagent is loaded to your plates and tubes. VisiBlue is very easy to use by simple addition to your favorite mastermix.

**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 entire RT-qPCR experimental workflow. Add the spike to the experimental sample and to a control based on water. Processing both samples exactly the same way – any inhibition in the experimental sample will impair the RT-qPCR resulting in higher Cq than of the control sample. TATAA Universal Spikes have a synthetic sequence that is not present in any known living organism. The Spike assay is exceedingly robust and is optimized for high sensitivity for inhibition. The Cq of the Spike assay also reflects losses during extraction, handling, transport, and storage of samples, including freeze-thaw events during RT-qPCR.
TATAA Biocenter, with offices in Gothenburg, San Francisco 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 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.