BOXTO A new dye for qPCR

Background

BOXTO is a derivative of BEBO, an unsymmetrical cyanine dye developed by TATAA Biocenter for use in qPCR applications. The dye has absorbance and emission wavelengths that can be detected on the JOE channel on most common real-time PCR instruments, and shows a very strong fluorescence increase when bound to dsDNA. BOXTO can be used as an unspecific dye for real-time PCR applications or other applications where staining of dsDNA is wanted. The BEBO-family of dyes is patent pending.

Best of both worlds

Since unspecific dyes also detect artefacts such as primerdimers, 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 gel electrophoresis has to be performed to troubleshoot leading to extremely high risk of contamination. BOXTO can be used in combination with any FAM-labelled 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.

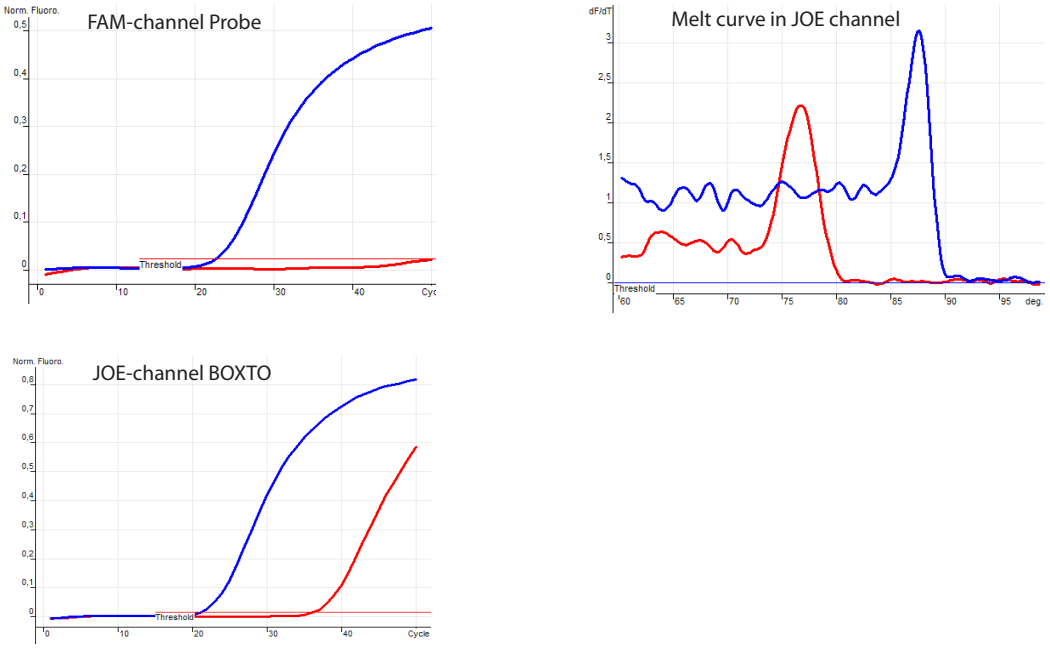

Figure: Using BOXTO in combination with a molecular beacon FAM-labelled probe. Detecting on the FAM-channel does not detect the NTC containing only primerdimers (red curves). These are detected in the JOE-channel with BOXTO (right) in the same reaction tube. Dissociation curve analysis can be performed after amplification using BOXTO on the JOE-channel. Here 0,7µM of BOXTO was used in combination with 0,4µM of the molecular beacon probe.

tataabiocenter

TATAA Biocenter AB
Medicinaregatan 8A, 413 46 Göteborg
Tel: +46 31 741 18 00, Fax: +46 31 741 17 01, E-mail: info@tataa.com

BOXTO A new dye for qPCR

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.

Properties in qPCR

BOXTO binds to the minor groove of dsDNA and the fl uorescence signal is proportional to the amount of DNA, making it suitable to use as a non-specifi c reporter in qPCR. Using standard reaction conditions and thermal profi les BOXTO can be added to achieve detection of any amplifi ed DNA.

BOXTO exhibits linearity of many orders of magnitude and lets the user make a dissociation curve after completed PCR for further information about the amplifi cation.

BOXTO has been demonstrated to function well on multiple instrument platforms and has been validated using several of the common commercially available mastermixes intended for probes. No optimization is necessary in most cases. BOXTO is simply added to the mix and used with standard thermal profi le. Alternatively, home-brew mastermixes can be used.

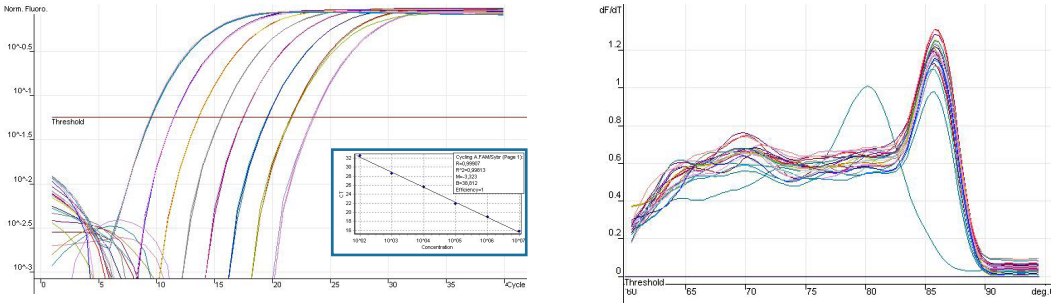

Figure : Amplifi cation curves of a serial dilution using BOXTO detection. BOXTO has been demonstrated to show linearity over many orders of magnitude and shows similar effi ciencies and better sensitivities compared to SYBR Green I. Dissociation curves allow the detection and discrimination of different amplifi cation products.

Storage and instructions for use

1µl of stock solution is supplied at 1.5mM in DMSO and is stable for several months in -20oC. Before use dilute the stock with 39µl of mQ water. A solution of 37.5µM (a 1+39 dilution) is stable for more than 1 month at 4oC. Typical concentration in qPCR reactions is approximately 0.5µM (concentrations around 0.3-0.7µM can be tested). Detection should be done on the JOE channel. The dye can be used with most commercially available qPCR reagents. The quality of signal may depend on the chemistry used and on the filter settings on the qPCR platform.

References

M. Bengtsson, J. Karlsson, G. Westman and M. Kubista (2003) A new minor groove binding asymmetric cyanine reporter dye for real-time PCR, Nucleic Acid Research, Vol. 31, No. 8, 1-5

H.J. Karlsson, P. Lincoln, G. Westman (2003) Synthesis and DNA Binding Studies of a New Asymmetric Cyanine Dye Binding in the Minor Groove of [poly(dA-dT)]2. Bioorganic & Medicinal Chemistry, 11, 1035-1040.

tataabiocenter

TATAA Biocenter AB
Medicinaregatan 8A, 413 46 Göteborg
Tel: +46 31 741 18 00, Fax: +46 31 741 17 01, E-mail: info@tataa.com