Top-Bio s.r.o.

Tel: +420 603 476 934
E-mail: top-bio@top-bio.cz
www.top-bio.com

qPCR Visible Blue Mark

(Cat. No. B129) rev. 11/2022

Description

The qPCR Visible Blue Mark is a dye enhancing the visibility of real-time PCR reaction mixtures, enabling easier loading of small-volume reactions into tubes or multi-well plates with greater speed, accuracy, and precision (Fig. 1). The presence of the dye is particularly advantageous for single nucleotide polymorphism (SNP) genotyping and high-throughput gene expression analysis using multi-well plates. The presence of the dye does not adversely affect the performance of the PCR reactions when used as directed; it does not interfere with widely used qPCR fluorescence dyes, such as SYBR Green, EvaGreen, or probe-based detections. It is optimized for various real-time PCR mixes of the Top-Bio provenience, including hot-start PCR mixes. The qPCR Visible Blue Mark can be used with various real-time PCR systems. For convenience, qPCR Visible Blue Mark can be added directly to the master mixes or used as a component of PCR mixes added at a selected step.

Technical data

Component and package

- A tube with 10 µl of 200x concentrated qPCR Visible Blue Mark, which delivers 1000 x 20 µl PCR reaction mixtures.

Storage

- When protected from light, the product can be stored at a temperature of -20°C ± 5°C for 12 months, for up to 1 month at 4°C ± 2°C, and several days at a temperature of up to 30°C.

Composition

- The qPCR Visible Blue Mark is a 200x concentrated non-toxic blue dye in 75 mM Tris-HCl, pH 8.0 (at 25°C), supplemented with stabilizers and additives.

Purity and quality control

- Each product batch is tested for performance in qPCR using SYBR Green as a fluorescence dye. No difference in qPCR performance is observed in the presence of the dye. A typical result of such testing is shown in Fig 2.

Cat.No.	Product name	Quantity
B129	qPCR Visible Blue Mark	100 µl

Protocol

The following protocol is a guideline and should be optimized according to specific applications. In the final qPCR, the qPCR Visible Blue Mark must be diluted 200x.

A. Addition of the qPCR Visible Blue Mark to the Master mix

1. Thaw 200x concentrated qPCR Visible Blue Mark and the recipient Master mix (e.g. qPCR 2x Blue Master mix, 500 µl, Top-Bio, Cat. No. P521) to room temperature.
2. Mix thoroughly and centrifuge briefly to collect the solution at the bottom of the tube. Keep the tube on ice, protected from light.
3. Add 5 µl of qPCR Visible Blue Mark into 500 µl of 2x concentrated master mix.
4. Mix thoroughly and centrifuge briefly to collect the solution at the bottom of the tube. Keep the tube on ice, protected from light.
5. For 20 µl PCR reaction, pipet 10 µl of the coloured qPCR master mix into a well of the multi-well plate, strip or tube, followed by 10 µl of remaining components (DNA, primers, probes). For loading clear tubes or plates, placing them on top of a white background is recommended to enhance visibility.

B. Addition of the qPCR Visible Blue Mark to the DNA samples

The reagent can be added to the DNA sample for tracking the addition of the sample to wells.

1. Thaw 200x concentrated qPCR Visible Blue Mark and the samples to track to room temperature.
2. Mix qPCR Visible Blue Mark thoroughly and centrifuge briefly to collect the solution at the bottom of the tubes. Keep the tubes on ice, protected from light.
3. Pipet the appropriate amount of the Blue Mark into the sample. Consider that qPCR Visible Blue Mark must be in the final PCR reaction diluted 200x. See examples in Table 1.

Table 1. *Recommended volumes (in µl) for adding qPCR Visible Blue Mark to DNA template.*

Final PCR Volume	DNA Template Volume per PCR	Sample Volume	200x qPCR Visible Blue Mark Added to Sample
20	4	100	2.5
20	2	20	1
10	2	100	2.5
10	1	20	1

Figure 1. *qPCR Visible Blue Mark facilitates pipetting into multi-well plates. Wells of the 384-well plate were pipetted with 10 µl aliquotes of qPCR 2xSYBR Master Mix (columns 3-8) or 10 µl aliquotes of qPCR 2xSYBR Master Mix_BLUE containing 100-fold diluted qPCR Visible Blue Mark (columns 1, 2, 9-14, 23, 24). In columns 16-22, qPCR 2xSYBR Master Mix_BLUE was pipetted only in rows A, C, E, G, I, K, M and O. In column 15, all wells were left blank.*

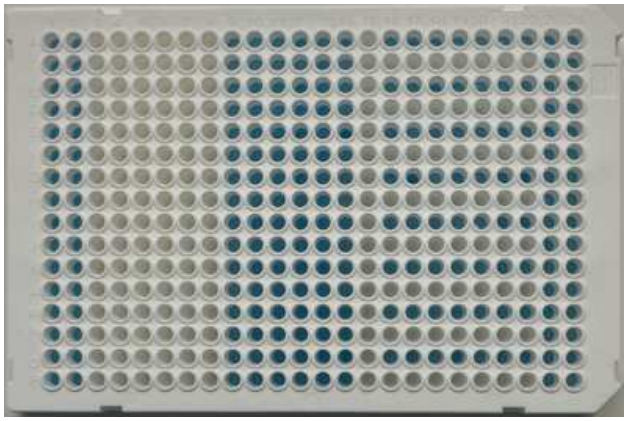

Figure 2. *qPCR Visible Blue Mark does not affect the qPCR performance. qPCR 2x Blue Master Mix (Top-Bio, Cat. No. P521) was supplemented with qPCR Visible Blue Mark (blue lines; final dilution 1:200) or not (green lines), SYBR Green 1, DNA template at five 10-fold dilutions, and the corresponding primers. No DNA template control (NTC) was also included. The samples in triplicates were analyzed by a real-time PCR cycler.*