FAQs for QPCR Master Mixes

https://www.biovision.com/products/molecular-biology-tools/pcr-rtpcr-qpcr-dna-polymerases/qpcr.html

1. **What is the difference between Jade™ Master Mixes (BV Cat# M1105-M1120) and SybrGreen? Which one has better features? What is the dye used in Jade™ Master Mix?**

   Jade™ Master Mixes (BV Cat# M1105-M1120) include a new generation of SybrGreen dyes, which give a stronger signal when bound to dsDNA as compared to SybrGreen. They also have reduced toxicity. Jade™ Master Mixes provide much higher sensitivity and better low-copy detection results.

2. **What are the QPCR Master Mixes available from Biovision?**

   BioVision offers multiple 2X QPCR Master Mixes for quantitative PCR analysis. Available with the option of ROX or fluorescein as the internal passive reference dye, 2X QPCR Master Mixes offers unparalleled performance in sensitivity, signal-to-noise ratio, and complete elimination of primer dimers. Due to the design variations of QPCR instruments from different manufacturers, BioVision's 2X QPCR Master Mix formulations are optimized for different QPCR instruments available in the market. Each Master Mix is preconfigured with a reference dye specific for a particular instrument. Thus, the customers get to choose what works best for their instrument while having the advantage of using a reference dye. Different companies optimized their instruments with different reference dyes (ROX, Fluorescein, etc). Please refer to BioVision's **QPCR Master Mix Selection Guide** in the link https://www.biovision.com/products/molecular-biology-tools/pcr-rtpcr-qpcr-dna-polymerases.html for selecting the appropriate QPCR formulations applicable to the end users particular instrument model.

3. **What is the difference between Jade™, JadeExpress™ and JadeSmart™ QPCR Master Mixes?**

   Jade™ Master Mixes (BV Cat# M1105-M1108) use a proprietary chemical modification of the DNA polymerase included in the Master Mix for hot start PCR, conferring a significant reduction in the non-specific PCR amplification. JadeExpress™ Master Mixes (BV Cat# M1109-M1112) use Breeze™ DNA polymerase (BV Cat# M1148) for ultrafast PCR, conferring a significant reduction in the overall QPCR quantification and detection time, thus streamlining the experiment through cost and labor saving. JadeSmart™ Master Mixes (BV Cat# M1113-M116) contain Fire Start™ DNA Polymerase (BV Cat# M1149) to amplify DNA targets with varying lengths, GC contents and to overcome DNA fragments that lead to problematic secondary structures.

4. What is JademiRNA™ Master Mix?

Jade miRNA™ Master Mixes (BV Cat# M1117-M1120) are designed specifically for quantitative real-time analysis of miRNA obtained from RNA samples. The Master Mix is also designed to eliminate primer-dimer formation and amplification of premature miRNAs. The chemically modified Hot Start Taq polymerase included in the Master Mix significantly reduces the non-specific PCR amplification observed with regular Taq polymerases.

5. Why are there four different kinds of QPCR Master Mixes under each of Jade™, JadeExpress™, JadeSmart™, JademiRNA™ and Taqman Master Mixes?

Jade™ (BV Cat# M1105-M1120) and Taqman Master Mixes (BV Cat# M1121-M1125) are available in a range of formulations, each of which has been carefully optimized for performance according to the make and model of the QPCR machine and the reference dye.

a. Mix (No dye), without a reference dye (ROX) dye,
b. icycler, for icycler system,
c. Low ROX, for low ROX
d. ROX, contains regular ROX

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6. Do we need to design or obtain the gene specific Taqman probes for Taqman Master Mixes (BV Cat# M1121-M1125)?

Yes, Taqman probes vary with end user's sample targets. Each Taqman probe is a sequence-specific probe with a reporter fluorescent dye at the 5' end and a quencher dye at the 3' end. To obtain Taqman probes, please contact your preferred vendor.

7. What is the detection dye in Taqman Master Mixes?

The Taqman probe will contain the detection dye. Each Taqman probe is a sequence-specific probe with a reporter fluorescent dye at the 5' end and a quencher dye at the 3' end.

8. What is Taqman Master Mix-Multiplex?

Taqman Master Mix-Multiplex (BV Cat# M1125) is designed for high throughput quantitative PCR using Taqman probe-based chemistry. Available with the option of ROX or fluorescein as the internal passive reference dye, Taqman 2X qPCR Master Mix offers superb performance in sensitivity and signal-to-noise ratio. The Multiplex formulation supports quantitative amplification and detection of up to four targets simultaneously with consistent and reliable results.

9. How to check the instrument compatibility with the QPCR Master Mixes available from BioVision?

We have a selection guide on BioVision website. Please refer to BioVision's **QPCR Master Mix Selection Guide** in the link https://www.biovision.com/products/molecular-biology-tools/pcr-rtpcr-qpcr-dna-polymerases.html for selecting the appropriate QPCR formulations applicable to the end users particular instrument model.

10. If I have to synthesize cDNA from RNA before qPCR, what cDNA synthesis kits do you recommend?

You can use cDNA Synthesis Kits available from BioVision (BV Cat# M1164-M1172) for cDNA synthesis from RNA.

11. Can I vortex the reagents?

Yes. The reagents can be vortexed.

12. What is the difference between the fluorescent detection dye & the reference dye used in the qPCR Master Mixes?

The detection dye (SybrGreen) binds to the DNA and emits fluorescence at a certain wavelength. It reports the presence and helps in the quantification of the amplified DNA. Reference dye (for example, ROX) is a fluorescent dye, which emits at a different wavelength from the detection dye's emission channel (no detection interference) and absorbs at a wavelength different from the emission channel of detection dye's (no signal quenching). The main purpose of reference dye is to normalize/correct the "well-to-well" variation of the instrument such as the difference in light paths between wells. The reference dye can also normalize the non-PCR-related fluctuations in fluorescence and provides a stable baseline for multiplex quantitative PCR and RT-PCR analysis.

13. Does your qPCR Master Mixes work for the FAST protocol?

All BioVision's qPCR Master Mixes (except for the Express line, BV Cat# M1109-M1112) work with the FAST protocol. The Express qPCR line protocol is even faster than the FAST protocol.

14. What are the main advantages of Jade™ Master Mixes?

Jade™ Master Mixes (BV Cat# M1105-M1120) provide high sensitivity in a ready-to-use format. Additionally, BioVision's QPCR Master Mix formulations are optimized for different QPCR instruments available in the market.

15. Can I use the Jade™ Master Mix for Taqman probe?

No, the formulations are not compatible. Please refer to Taqman Master Mix series (BV Cat# M1121-M1125).

16. Do your qPCR Master Mixes contain Uracil-DNA glycosylase?

No, qPCR Master Mixes do not contain Uracil-DNA glycosylase.

17. What is the concentration of $MgCl_2$ in the Jade™ Master Mix and Taqman Master Mixes?

The concentration of $MgCl_2$ is 6 mM in 2X Master Mixes. The final concentration of $MgCl_2$ in qPCR reaction is 3 mM.

18. The protocol of Biovision's qPCR Master Mixes states a 2 step thermal cycle instead of a normal 3 steps program, is it a mistake in the protocol?

The protocol is correct. A 2-step thermal cycle program is very common in qPCR and the results are equivalent to those obtained from a conventional 3 steps thermal cycle qPCR experiment but only using a fraction of the time. Biovision's qPCR Master Mixes are fully compatible with both quick 2 steps thermal cycle and conventional 3 steps thermal cycle programs.

19. Usually a SYBR dye doesn't require calibration. Does Jade™ Master Mixes require calibration?

Jade™ Master Mix does not require calibration.

20. Are Jade™ Master Mixes suitable for experiments being setup under normal lighting?

Jade Master Mixes™ are very stable and are suitable for setting up experiments under normal lighting.

21. I noticed clear crystals in the qPCR Jade Master Mix solution (BV Cat# M1105). Is it still functional?

The qPCR Master Mixes should still be functional. The crystals are from our proprietary buffering salt, which will dissolve when you warm the solution up to room temperature or at 37°C. All our qPCR Master Mixes are very stable and can withstand this kind of warming cycle.

22. Is it correct that the T_m of the primers should be around the same as the annealing temp (~60°C)?

Yes, in qPCR having a T_m close to the annealing temperature will allow more specific priming. In turn, this may lead to a reduced yield when compared to PCR. The amplified end product will be more accurate and specific to the intended target.

23. What annealing temperature should I design for my primers? If the annealing temperature is lowered to 55°C, will this affect the efficiency of the polymerase?

We recommend designing primers with a T_m of 58-60°C, because the qPCR will be running at 60°C for both annealing and extension. A lower annealing temperature can be used, but you may still need to consider using 60°C for extension, thus using a 3 steps thermal cycling. A lower annealing temperature would also allow non-specific priming to occur.

24. We used a higher DNA concentration than what was recommended in the protocol and observed a flat curve and no CT value. Is this due to an overdose of DNA template?

It is called "template inhibition" and is a common phenomenon in PCR/qPCR reactions. When too much template is added, the templates can interact and bind to the Mg^{2+} ions, or the primers in the reaction mixture.

25. For BioRad iCycler systems, is the Jade Mastermix™ compatible with "dynamic" well factor calibration or a "persistent" well factor calibration run is needed?

JadeSmart™ Mix-iCycler (BV Cat# M1114) is specifically formulated for BioRad iCycler systems and it is fully compatible with dynamic well factor calibration.

Please refer to our QPCR Master Mix Selection Guide https://www.biovision.com/products/molecular-biology-tools/pcr-rtpcr-qpcr-dna-polymerases.html for selecting the appropriate QPCR formulations applicable to your particular instrument model.

26. Does Jade™ Mix-Low ROX (BV Cat# M1111) work for ABI7500 Fast?

Yes, Jade™ Mix-Low ROX (BV Cat# M1111) will work for ABI7500.

27. Can I use my own primers with Biovision's qPCR MasterMixes?

Yes, any primers can be used with BioVision's qPCR Master Mixes. Please make sure that the correct thermal cycling conditions for the primers are applied.

28. What is the concentration of dNTP in the qPCR Master Mixes?

Unfortunately, we cannot disclose the concentration of dNTP in the qPCR Master Mixes.

29. Can the qPCR Master Mixes be thawed in the fridge a few days ahead of time?

Thawing the qPCR Master Mix in the fridge without immediate mixing could affect the Master Mix composition. When the Master Mix is thawed but not mixed, the Master Mix is essentially left in a non-homogenized state where the kit components could potentially get precipitated. Thorough mixing is highly recommended after the Master Mix is thawed. Users can mix the Master Mix by flicking the tube a few times during the thawing process), and then store the thoroughly-mixed homogenized Master Mix in the fridge for short-term storage.

30. What is the Sanger miRBase Sequence Database?

miRBase is a searchable sequence database of published miRNA, that has been established by the Sanger Institute. Each entry in the microRNA Registry represents a predicted hairpin portion of a microRNA transcript, termed mir in the database with information on the location and sequence of the mature microRNA sequence (termed miR). The database provides microRNA gene hunters with unique names for novel microRNA genes prior to publication of results.

31. Do I need to use an internal control primer for quantitative detection of miRNAs, the same as housekeeping primers which we use in qPCR for mRNAs detection?

For human miRNA, the controls are as follows: 1)SNORD44 2)SNORD47 3)SNORD48 4) U6-2

32. Can I quantify mature miRNA in total RNA using your cDNA synthesis and kit and qPCR Master Mix?

Yes, you can quantify miRNA in total RNA using cDNA synthesis kit and qPCR Master Mixes.

33. How to search genes targeted by a specific miRNA?

You can search for the predicted and validated miRNA target genes at http://mirbase.org/. Enter miRNA name or accession number in the search bar and look for the targeted genes.

34. Does the profiling (qPCR) require a specific instrument or can it be done by every real time PCR instrument?

We have a range of qPCR mastermixes (BV Cat# M1105-M1120) available in optimized formulations for all qPCR machines. Please refer to BioVision's **QPCR Master Mix Selection Guide** in the link https://www.biovision.com/products/molecular-biology-tools/pcr-rtpcr-qpcr-dna-polymerases.html for selecting the appropriate QPCR formulations applicable to the end users particular instrument model.

35. Can I use SYBR green Master Mix for downstream qPCR of the cDNA produced from miRNA using Novo™ Transcriptome cDNA Kit (BV Cat# M1167)?

You can use a SYBR green Master Mix for qPCR. However, our JademiRNA™ QPCR Master Mixes (BV Cat# M1117-1120) recipe has been optimized to provide the most successful conditions for qPCR. We have extensively tested minor changes in the composition of miRNA qPCR buffer. This has given very dramatic differences in qPCR results. General qPCR Master Mixes available have more additives to prevent non-specificity and formation of primer dimers and the conditions are often too harsh for the miRNA primers to anneal properly.

36. Can I use the JademiRNA™-ROX™ Mix-ROX (BV Cat# M1120) on longer RNA strands, such as mRNA?

The miRNA qPCR Master Mixes are not suitable for mRNA amplification. It is better to use our regular qPCR Master Mix product line for mRNA.

37. What is the solution used to dilute the cDNA template in BV Cat# M1126?

Use nuclease free water to dilute the cDNA template.