

# Precision Blue™ Real-Time PCR Dye

Catalog #	Description
172-5555	<b>Precision Blue Real-Time PCR Dye</b> , 55 µl (1 x 1 ml vial), 200x concentrate, sufficient for 5 ml of 2x supermix

For research purposes only.

## Storage and Stability

Guaranteed for 12 months in a constant temperature freezer at –20°C when protected from light. For convenience, this reagent can be stored at 4°C when protected from light for up to 3 months.

## Kit Contents

Precision Blue real-time PCR dye is a 200x concentrated, ready-to-use, inert reagent that enhances the visibility of real-time PCR reactions for more accurate pipetting, plate loading, and reaction tracking. It will not adversely affect fluorescence or performance when used as directed and is optimized for use with all Bio-Rad universal real-time PCR supermixes and kits.

## Instrument Compatibility

This reagent has been validated on Bio-Rad’s CFX96™, CFX384™, CFX96 Touch™, CFX384 Touch™, and CFX Connect™ real-time PCR detection systems and has been tested for compatibility on ROX-dependent Life Technologies real-time PCR instruments. This reagent may also be compatible with other systems but should be validated by the user to ensure optimal performance.

## Reagent Compatibility

Precision Blue real-time PCR dye has been validated for use with the Bio-Rad real-time PCR supermixes and kits listed in Table 1.

**Table 1. Compatibility of Precision Blue dye with Bio-Rad real-time PCR reagents.**

SYBR® Green Chemistry	Probe Chemistry (FAM, HEX, or VIC channel)
SsoAdvanced™ universal SYBR® Green supermix	SsoAdvanced universal probes supermix
iTaq™ universal SYBR® Green supermix	iTaq universal probes supermix
iTaq™ universal SYBR® Green one-step kit	iTaq universal probes one-step kit

This reagent has also been tested for compatibility with Bio-Rad’s iQ™ SYBR® Green supermix and SsoFast™ EvaGreen supermix on Bio-Rad instruments.

## Reagent Use Instructions

### Option 1: Addition to Bio-Rad Universal Supermixes or One-Step Kits

For ease of use, we recommend that Precision Blue dye be added directly to Bio-Rad universal supermixes or the 2x reaction mix of the iTaq universal one-step kits and be used immediately or stored according to supermix directions.

1. Thaw Precision Blue and the recipient mix to room temperature.
2. Mix thoroughly, centrifuge briefly to collect the solutions at the bottom of the tubes, then store on ice protected from light.
3. Pipet 11 µl of Precision Blue into 1 ml of the Bio-Rad 2x universal supermix or one-step kit reaction mix.
4. Mix thoroughly, centrifuge briefly to collect the solution at the bottom of the tube, then store on ice protected from light. Continue by following the respective reagent protocol.
5. For subsequent loading of clear tubes or plates, it is recommended to place them on top of a white background to enhance visibility.

### Option 2: Addition to PCR Reaction Master Mix

Precision Blue dye can be used as a component of PCR reaction master mixes, such as a 1x PCR supermix and template mixture. This method is particularly useful for quick and accurate single-step pipetting of reaction mixes into Bio-Rad’s PrimePCR™ assay plates (Figure 1).

1. Thaw all reagents, including Precision Blue, to room temperature.
2. Mix thoroughly, centrifuge briefly to collect the solutions at the bottom of the tubes, then store on ice protected from light.
3. Prepare the master mix and scale the amount of Precision Blue proportionally to the amount of 2x supermix used (1 µl 200x Precision Blue per 100 µl supermix).
4. Mix the assay master mix thoroughly to ensure homogeneity and dispense equal aliquots into each PCR tube or plate well.

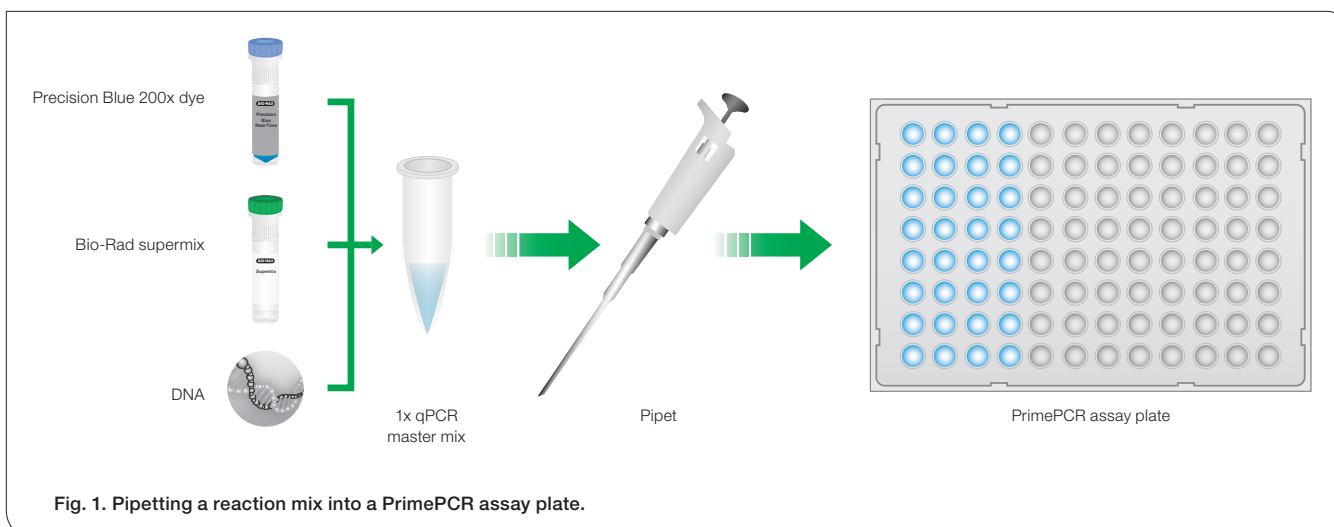


Fig. 1. Pipetting a reaction mix into a PrimePCR assay plate.

### Option 3: Addition Directly into a DNA or RNA Sample

Precision Blue dye can be added to the sample for tracking the addition of just the sample to a well. This workflow option is designed for a single-use application and it is recommended to color only the amount of template necessary for the experiment because Precision Blue real-time PCR dye has not been validated for extended sample storage.

1. Thaw Precision Blue and the sample(s) to room temperature.
2. Mix Precision Blue thoroughly, centrifuge briefly to collect it at the bottom of the tube, then store on ice protected from light.
3. Pipet the appropriate amount of Precision Blue into the sample. This amount will depend on template sample volume that is being mixed, template amount that will be added to each real-time reaction, and the final volume of the real-time reaction, in order to yield a 1x dye concentration in the final reaction. See Table 2 for suggestions.
4. After adding the dye, mix thoroughly, centrifuge briefly to collect the solution at the bottom of the tube, then store on ice protected from light. The template sample may now be pipetted into the reaction wells.

Table 2. Recommended volumes for adding dye to a nucleic acid sample.

Final Reaction Volume, $\mu$ l	Template Loaded per PCR Reaction, $\mu$ l	Sample Volume, $\mu$ l	200x Dye Volume Added to Sample, $\mu$ l
20	4	100	2.5
10	2	100	2.5
20	2	20	1
10	1	20	1

To learn more about sample preparation, assay and experimental design (including duplex optimization), and troubleshooting, visit [www.bio-rad.com](http://www.bio-rad.com) and search for items **10031339** and **10031340**.

### Quality Control

Precision Blue real-time PCR dye enables enhanced pipetting precision and reaction mix visibility without affecting PCR performance. Stringent specifications are maintained to ensure lot-to-lot consistency. This product is free of detectable DNase and RNase activities.

### Related Products

Real-time PCR supermixes for quantitative PCR (qPCR):

- SsoAdvanced™ universal SYBR® Green supermix (172-5270)
- SsoAdvanced universal probes supermix (172-5280)
- iTaq™ universal SYBR® Green supermix (172-5120)
- iTaq universal probes supermix (172-5130)

Real-time PCR one-step kits for qPCR:

- iTaq™ universal SYBR® Green one-step kit (172-5150)
- iTaq universal probes one-step kit (172-5140)

To learn more about Bio-Rad's complete solution for amplification, visit [www.bio-rad.com/amplification](http://www.bio-rad.com/amplification).

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Bio-Rad's real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

Practice of the patented 5' Nuclease Process requires a license from Applied Biosystems. The purchase of iQ, iTaq, SsoAdvanced, and SsoFast supermixes includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research when used with the separate purchase of Licensed Probe. No other patent rights are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained from the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.