5X PrimePath™ Probe qPCR Kit, ROX plus, GPR Protocol-At-A-Glance

I. Introduction

5X PrimePath™ Probe qPCR Kit, ROX plus, GPR (Cat. Nos. 638348 & 638350) enables the user to perform accurate qPCR on DNA samples. The highly concentrated master mix allows for flexibility in handling larger sample volumes. The entire protocol from setup to detection takes less than one hour, enabling fast results.

II. Required Materials

This protocol applies to the following Takara Bio products:

- 5X PrimePath Probe qPCR Kit, ROX plus, GPR (Cat. Nos. 638348 & 638350)

Additional materials required:

- Primers and probes
- Micropipette and tips (with hydrophobic filters)
- Vortex mixer
- Benchtop centrifuge for tubes or plates
- 1.5 ml Eppendorf tubes, 200 µl PCR tubes, or 200 µl PCR plates for sample preparation
- Tubes or plates for real-time PCR
- A real-time PCR machine compatible with **low ROX** (e.g., QuantStudio 3 or later, ABI 7500, ABI 7500 Fast, ViiA 7, Stratagene MX4000P, MX3000P, MX3005P, etc.)

NOTE: If using a high ROX qPCR instrument, add ROX based on the guidelines for your model and the concentration of the ROX additive used.

III. Protocol

1. Mix the tube of 5X PrimePath Probe qPCR Mix by inverting several times then spin down for 10 sec; the mix may appear cloudy after storage, but this does not affect its performance.
2. On ice and using the table below, combine everything for the qPCR reaction mix EXCEPT the DNA sample for all planned reactions, plus 10% of the total reaction mix volume for overage.

NOTES:
- If the mixture is used immediately, it can be combined at room temperature (no ice).
- Do not add the sample at this time. It is listed in the table to indicate what the final mixture will contain.

qPCR reaction mix (per 25 µl* reaction)

Reagent	Final concentration	Singleplex	Multiplex (*N*† targets)
5X PrimePath Probe qPCR Mix, ROX plus, GPR	1X	5 µl	5 µl
PCR Forward Primer (10 µM)	0.2 µM	0.5 µl	*N*† x 0.5 µl
PCR Reverse Primer (10 µM)	0.2 µM	0.5 µl	*N*† x 0.5 µl
Probe (10 µM)	0.2 µM	0.5 µl	*N*† x 0.5 µl
DNA sample‡	—	2 µl§	2 µl§
RNase Free H_2O	—	Up to 25 µl**	Up to 25 µl**
Total volume per reaction		**25 µl**	**25 µl**

*Mix, as written, can also be used per 20 µl reaction.

†Where *N* represents the total number of targets of interest. The total reaction volume will still be 25 µl, while 0.5 µl of each primer and probe will be added per target.

‡Do not add to the reaction mix; this will be added to the mix in Step 4.

§The most common sample volume of Takara Bio PCR products. It is possible to add more; however, depending on the chemicals contained in the sample solution, higher sample volume may have adverse effects.

**Adjust the reaction volume according to the recommendations for the real-time PCR instrument used.

3. Add [25 µl – Volume of DNA Sample] of the qPCR reaction mixture into a PCR tube or a 96-well PCR plate.
4. Dispense the volume of DNA sample into the PCR tube or plate well. Spin down for 10 sec.
5. Set up the thermal cycler and run the assay using the reaction conditions below:

95°C	20 sec
40 cycles:	
95°C	1 sec
60°C	20 sec

NOTE: Please follow the instruction manual of the real-time qPCR machine used. If the default setting does not work, perform manual analysis per the instruction manual.

6. After the reaction is complete, check the amplification curve. Confirm that the analytical parameters are correct and that the Ct value has been calculated.

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