5X One Step PrimeScript™ III RT-qPCR Kit, ROX plus, GPR Protocol-At-A-Glance

I. Introduction

5X One Step PrimeScript™ III RT-qPCR Kit, ROX plus, GPR enables the user to perform accurate, one-step RT-qPCR on RNA samples. The highly concentrated master mix allows for flexibility in handling larger sample volumes. The entire protocol to detection takes less than one hour, enabling fast results.

II. Required Materials

This protocol applies to the following Takara Bio products:

- 5X One Step PrimeScript III RT-qPCR Kit, ROX plus, GPR (Cat. Nos. 638336 & XU0001)

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.)

III. Protocol

1. On ice, prepare the RT-qPCR reaction mix for all reactions of the RNA sample, plus 10% of the total reaction mix volume for overage, using the table below.

   Before adding the 5X One Step PrimeScript III RT-qPCR Mix, mix the contents of the tube by inverting several times and then spin down; the mix may appear cloudy after storage, but this does not affect its performance.

   Use the reaction mix immediately.

NOTES:

– The RNA sample should not be added to the reaction mix; it will be added during Step 3.

– Refer to the "5X One Step PrimeScript III RT-qPCR Kit, ROX plus, GPR User Manual" for more information on calculating the overage.

RT-qPCR reaction mix (per 25 µl reaction)

Reagent	Final concentration	Singleplex	Multiplex (*N** targets)
5X One Step PrimeScript III RT-qPCR Mix, ROX plus, GPR	1X	5 µl	5 µl
PCR Forward Primer (10 µM)	0.2 µM†	0.4 µl	*N** x 0.4 µl
PCR Reverse Primer (10 µM)	0.2 µM†	0.4 µl	*N** x 0.4 µl
Probe (10 µM)	0.2 µM†	0.4 µl	*N** x 0.4 µl
RNA 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**

*Where *N* represents the total number of targets of interest. The total reaction volume will still be 25 µl, while 0.4 µl of each primer and probe will be added per target.

†For more information about primer, probe, and sample concentrations, please refer to the user manual.

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

2. Add [25 µl – Volume of RNA Sample] of the reaction mixture into a PCR tube or a 96-well PCR plate.
3. Dispense the volume of RNA sample into the PCR tube or plate well.
4. Set up the thermal cycler and run the assay using the reaction conditions below:

52°C	5 min
95°C	10 sec
40 cycles:	
95°C	5 sec
60°C	30 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.

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

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