I. Introduction

Lyo-Ready PrimePath™ Probe qPCR Mix, GPR (Cat. No. 638352) enables the user to perform accurate probe-based qPCR on purified DNA samples using a lyophilized master mix. Primers and probes can be added to the mix prior to lyophilization. Once dried, the master mix allows for flexibility in handling larger sample volumes, in addition to the convenience of room-temperature storage and transport.

II. Required Materials

This protocol applies to the following Takara Bio products:

- Lyo-Ready PrimePath Probe qPCR Mix, GPR (2X)*

  *Store at –20°C.

Additional materials required:

- Lyophilizer (e.g., SP VirTis Genesis Pilot Freeze Dryer, SP VirTis Ultra Pilot-Production Freeze Dryer [ATS Automation Tooling Systems, Inc.], etc.)
- 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 with optical seals or caps
- A real-time PCR machine (e.g., CFX96 Real-Time PCR Detection System [Bio-Rad], QuantStudio 3 or 5 [Thermo Fisher Scientific])

III. Protocol

A. Lyophilization

The master mix can be lyophilized in concentrations from 1X to 2X and can include primers and probes (i.e., assay). Lyophilization recipes will vary based on individual needs. Optimization will be required.

- Total cycle time and drying times will vary; 15–24+ hours can be expected.
- Long-term storage at ambient temperatures requires that the lyophilized product be packaged in a heat-sealed pouch with silica desiccant and at low relative humidity conditions.

Example:

1. Dispense 10 µl Lyo-Ready PrimePath Probe qPCR Mix, GPR into each tube of an 8-well strip in a 96-well rack.
2. With the lids open on the tubes, place racks into a lyophilizer.
3. Freeze at –70°C for 4 hr.
4. Dry at –30°C, 150 mTorr until primary drying is complete (≥6.5 hr).
5. Perform secondary drying at 20–25°C.

SAFE STOPPING POINT: Lyophilized cakes can be stored long-term following the parameters above.

B. qPCR Reaction Mix Preparation

1. Prepare the reaction mix for the qPCR reaction as illustrated below (optimization is recommended).

   Examples:

   - **qPCR reaction mixture (1 rxn) for cakes lyophilized with an assay**

1 cake	Lyophilized PrimePath Probe qPCR Mix + primers/probes
18 µl	DNase/RNase Free H_2O
2 µl	DNA sample*
20 µl	**Total volume**

   - **qPCR reaction mixture (1 rxn) for cakes without an assay**

1 cake	Lyophilized PrimePath Probe qPCR Mix
1 µl	20X primer/probe mix (provided by user)
17 µl	DNase/RNase Free H_2O
2 µl	DNA sample*
20 µl	**Total volume**

   *The DNA range will be dependent on your individual assay and testing conditions. You will need to optimize the volume for your experimental parameters.

2. Vortex until cake is completely dissolved then spin down.
3. Wait 5 min, then repeat Step 2.

NOT A SAFE STOPPING POINT: Proceed to the qPCR reaction protocol (Section C).

C. qPCR Reaction Protocol

1. Run the standard cycling protocol according to the instruction manual of your real-time qPCR instrument.

   The example PCR program below was used for detecting a multiplex assay of five targets (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, an spike-in control, and Human RNaseP target) and high-quality results were obtained.

   NOTE: Further optimization may be required for your experiment.

   Example:

95°C	2 min		Initial denaturation
40 cycles:			
95°C	10 sec	⎫	Denaturation
60°C	30 sec	⎭	Anneal, extend, and capture

2. After the reaction is complete, check the amplification curve. Confirm that the analytical parameters are appropriate and that the Cq value has been calculated.

   NOTE: If the default setting analysis does not work, perform manual analysis per the instrument's instruction manual.

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