# I. Introduction

**Lyophilized PrimePath™ Probe qPCR Mix** (Cat. No. 638351) enables the user to perform accurate probe-based qPCR on purified DNA samples using a lyophilized master mix. The cakes allow 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:

- Lyophilized PrimePath Probe qPCR Mix* 3 x 8-tube strips

  *Store at room temperature. Store opened, unused cakes in a desiccator.

**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 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. qPCR Reaction Mix Preparation

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

   **Example:**

   - **qPCR reaction mixture (1 rxn)**

| | |
|---|---|
| 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 the 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 B).

## B. 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,* a 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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