Notices

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Product in development. This protocol may serve as an initial guide for evaluation but may not be fully optimized. IDT does not guarantee the performance of this protocol, as development is in progress and verification testing has not been completed.

Safety notices

Reminder symbols call attention to minor details that may be easily overlooked and compromise the procedure resulting in decreased assay performance.

Caution symbols denote critical steps in the procedure where risk of protocol failure or damage to the product itself could occur if not carefully observed.

Stop symbols indicate where this procedure may be safely suspended and resumed at a later time without risk of compromised assay performance. Make note of these steps and plan your workflow accordingly.

Revision history

Version	Release date	Description of changes
01		*Initial revision*

Contents

Technical support

Visit https://www.idtdna.com/pages/support for a helpful answers to frequently asked questions or contact us directly at applicationsupport@idtdna.com.

Overview

Intended use

This protocol describes a workflow for the amplification of PrimeTime™ Research Panels primer/probe mixes in both purified and crude specimens using the PrimeTime™ Research Panels and the PrimeTime™ 1-Step 4X Broad-Range Master Mix. The intended use of this product and associated protocol is for **Research Use Only (RUO)**.

The primer/probe mixes, control, master mix and its associated components should be stored at, or below, -20°C.

Materials Supplied		
Description	**Part Number**	**Quantity**
PrimeTime™ Research Panel Primer/Probe Mix	Various	500 rxn
PrimeTime™ Research Pathogen Universal Control	10028571	300 µl
PrimeTime™ One-Step 4X Broad-Range Master Mix	Various	1-25 ml

Additional materials required to perform this protocol, but not supplied in the PrimeTime™ Research Panel primer/probe mixes

Materials Required, but not Supplied		
Description	**Supplier**	**Part Number**
Nuclease Free Water	Various	-
10 mM Tris, 0.1 mM EDTA solution, pH 7.5	Various	-
Extraction Kit/Protocol for your sample type*	Various	-

* - The PrimeTime™ DA Enhancer reagent (Included with the PrimeTime™ One-Step 4X Broad-Range Master Mix) may be added to the reaction to allow for the direct amplification of the targets from crude or unextracted samples.

Before getting started

Important precautions

- Read through the entire protocol before starting your qPCR reaction.

- Universal laboratory safety precautions should be followed for all testing outlined within this protocol as well as any laboratory specific safety protocols.
- The PrimeTime™ 1-Step 4X Broad-Range Master Mix reagent is heat sensitive. It is recommended to:
    - Thaw the Master Mix at 4°C or on ice. The product should remain liquid at the recommended -20°C storage condition, but temperature fluctuations may cause the product to freeze solid (which is normal).
    - Avoid repeated freezing and thawing.

Reagent storage and handling

- All reagents included in the PrimeTime™ Research Panel primer/probe mixes, PrimeTime™ Research Pathogen Universal Control and PrimeTime™ One-Step 4X Broad-Range Master Mix should be stored at -20°C and thawed at 4°C or on ice.
- The PrimeTime™ 1-Step 4X Broad-Range Master Mix reagent is heat sensitive. It is recommended to avoid repeated freezing and thawing cycles.
    - **Note**: The Master Mix should remain liquid at -20°C, but temperature fluctuations may cause the product to freeze solid (which is normal).

Preparation of the PrimeTime™ Research Pathogen Universal Control

- Once thawed, it is recommended to dilute the PrimeTime™ Research Pathogen Universal Control in a 10 mM Tris, 0.1 mM EDTA solution, pH 7.5.
- Perform a 1:100 dilution from the stock material to create a final positive control solution at 1000 copies/µL.
- The final positive control solution should be kept on ice until it is added to the reaction.

Protocol

Sample extraction (optional)

1. For purified and/or extracted specimen samples:
    a. Perform your desired extraction protocol to extract template for your given sample type.
2. For crude or unextracted specimen samples:
    a. Some sample types may be able to be directly amplified in the RT-qPCR reaction with the addition of the PrimeTime™ DA Enhancer reagent to the reaction.
    b. If attempting to directly amplify template from a compatible crude specimen sample type (e.g., blood, urine, saliva, or nasopharyngeal matrix) proceed to the next section.

Prepare the reaction mix

1. Determine the number of reactions needed for your experiment. Include the number of samples, replicates, control (e.g., no template control, positive control, etc.), and an appropriate excess overage amount to account for pipetting variability (e.g., 10%).
2. Combine the following reagents on ice based on the specimen sample type used:
    a. Singleplex reaction
        i. **For purified and/or extracted specimen samples**:

Component	Volume (µL)
PrimeTime™ One-Step 4X Broad-Range Master Mix	5
PrimeTime™ Research Panel Primer/Probe Mix	1
Sample or Control	1 - 5
Nuclease-Free Water	9 - 13
Total	**20**

        ii. **For crude or unextracted samples**:

Component	Volume (µL)
PrimeTime™ One-Step 4X Broad-Range Master Mix	5
PrimeTime™ Research Panel Primer/Probe Mix	1
PrimeTime™ DA Enhancer	1 - 2
Sample or Control	1 - 5

Nuclease-Free Water	7 - 12
Total	**20.0**

b. 4-plex reaction

i. **For purified and/or extracted specimen samples**:

Component	Volume (µL)
PrimeTime™ One-Step 4X Broad-Range Master Mix	5
PrimeTime™ Research Panel Primer/Probe Mix (FAM)	0.6*
PrimeTime™ Research Panel Primer/Probe Mix (SUN)	0.6*
PrimeTime™ Research Panel Primer/Probe Mix (TexRd)	0.6*
PrimeTime™ Research Panel Primer/Probe Mix (Cy5)	0.6*
Sample or Control	1 - 5
Nuclease-Free Water	7.6 – 11.6
Total	**20**

ii. **For crude or unextracted samples**:

Component	Volume (µL)
PrimeTime™ One-Step 4X Broad-Range Master Mix	5
PrimeTime™ Research Panel Primer/Probe Mix (FAM)	0.6*
PrimeTime™ Research Panel Primer/Probe Mix (SUN)	0.6*
PrimeTime™ Research Panel Primer/Probe Mix (TexRd)	0.6*
PrimeTime™ Research Panel Primer/Probe Mix (Cy5)	0.6*
PrimeTime™ DA Enhancer	1 - 2
Sample or Control	1 - 5
Nuclease-Free Water	6.6 – 10.6
Total	**20.0**

* Represents a starting volume of each primer/probe mix when multiplexing to 4-plexes. The final volume of each mix will be determined experimentally during multiplex optimization.

Run the RT-PCR

1. Place the plate in a real-time PCR instrument star the cycling program shown below.

Step	Temperature (°C)	Time	# of Cycles
1	50	15 minutes	1
2	95	3 minutes	1
3	95	15 seconds	40
4	60	1 minute	

2. Find the safety data sheets (SDSs) and certificates of conformance (COCs) for IDT products at:
    a. https://www.idtdna.com/pages/support/safety-data-sheets
    b. https://www.idtdna.com/pages/support/certificate-of-analysis-search
3. For additional information, Contact us.