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# FS-T-77241 2x Universal Fast Taqman Probe Multiplex qPCR Master Mix

## Description

2x Universal Fast TAQMAN Probe qPCR Master Mix is a Two-step Master Mix designed for multiplex real-time q-PCR using probe-based detection.
Easy to use: simply add template, primers, and probes.
This Master Mix includes antibody-modified Fast Hotstart and optimized PCR buffer which offers superior sensitivity, high accuracy, wide quantificable range: single & duplex reactions. Optimized for Multiplexing
The reagent incorporates a dUTP/UDG which prevent carry over-contamination .
Fast Taqman Probe Mix has been developed to retain a high level of performance in preassembled PCR reactions for up to 24 hours at Room Temperature.

## Kit Contents

| Contents | CAT. N° | Size |
|---|---|---|
| 2X Universal Fast TaqMan Probe Multiplex qPCR Master Mix | **FS-T-77241** | **5 X 1ML** |

***Target Specificity:*** *cDNA or gDNA*

**Applications:**

- *Gene Expression analysis,*
- *DNA Quantification /presence-absence*
- *Viral Plasmid DNA templates*
- *Probe Low-copy gene detection*
- *Fast Multiplex probe qPCR*
- *GC-Rich templates, complex templates*
- *mi-RNA Analysis*

**Features**

*• Ready-to-use 2X Master Mix• Fast, Multiplex & Highly Sensitive• Built-in Contamination Control*

### *1. PCR set up*

Primers and template can be prepared at room temperature, and the prepared PCR reaction system can be stored at room temperature for 24 hours without loss of amplification efficiency. Add the following reagents to the proper thermal cycler reaction tube or plate: Bring all components to room temperature and mix gently before dispensing.

## Reaction Conditions (10 µl reaction volume)

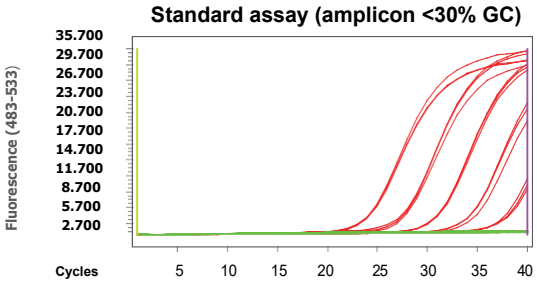
| Reagents | 10 µl Rnx Vol. | Final Concentration |
|---|---|---|
| 2X Universal Fast TaqMan Probe | 5 µl | 1X |
| Probe (10 µm)* | 0,4 µl | 0,1-0.5 µM(2) |
| (10 µm) Forward Primer*(1) | 0,2 µl | 0.2 µM |
| (10 µm) Reverse Primer*(1) | 0,2 µl | 0.2 µM |
| DNA Template** | 0.8 µl | - (3) |
| Rox Ref. Dye* | As required* | - |
| Water RNase Free | Up to 10 µl | - |

## Reaction Conditions (20 µl reaction volume)

| Reagents | 20 µl Rnx Vol. | Final Concentation |
|---|---|---|
| 2X Universal Fast TaqMan Probe | 10 µl | 1X |
| Probe (10 µm)* | 0,8 µl | 0,1-0.5 µM(2) |
| (10 µm) Forward Primer* (1) | 0,4 µl | 0.2 µl |
| (10 µm) Reverse Primer*(1) | 0,4 µl | 0.2 µl |
| DNA Template** | 1.6 µl | - (3) |
| Rox Ref. Dye | As required* | - |
| Water RNase Free | Up to 20 µl | - |

## Reaction Conditions (25 µl reaction volume)

| Reagents | 25 µl Rnx Vol. | Final Concentation |
|---|---|---|
| 2X Universal Fast TaqMan Probe | 12.5 µl | 1X |
| Probe (10 µm)* | 1 µl | 0,1-0.5 µM(2) |
| (10 µm) Forward Primer* (1) | 0,5 µl | 0.2 µl |
| (10 µm) Reverse Primer*(1) | 0,5 µl | 0.2 µl |
| DNA Template** | 2 µl | - (3) |
| Rox Ref. Dye | As required* | - |
| Water RNase Free | Up to 20 µl | - |



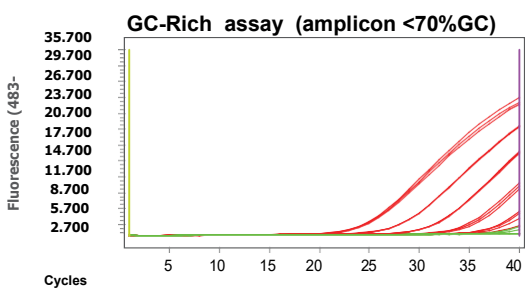




**Fig.1 & 2**: Universal Fast Taqman Probe 2X Multiplex qPCR Master Mix has been designed to deliver shorter number of cycles (40 cycles). PCR Reactions were performed using a dilution series from 100ng to 0.01ng of template cDNA. The amplification plots shows the superiority of the Fast Taqman Probe.

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**Note:**

***(1)** The optimal range for primers is 0.1~1.0 μM.
The primers with a final concentration of 0.2 μM work well.

**(2)** The **concentration of the probe** used is related to the Real Time PCR instrument, probe species and types of fluorescent label. Please refer to the instructions when using it.Typically, the final concentration is between 0.1 and 0.5 μM.

**(3) The DNA template** volume may be kept constant at 1–2 μL according to the template concentration, with the water volume adjusted to keep the total volume unchanged.

**See instructions for the use of** ROX dye and Instruments compatibility.

## 2 .Setup the plate

Transfer the reaction mixture to PCR tubes/plates. Reaction volumes can be reduced to10 μl if the instrument supports a low volume system. Cap or seal the reaction tubes/plates then centrifuge briefly to spin down the contents and eliminate any air bubbles.

## 3. Perform qPCR

Set the thermal cycling conditions using default PCR thermal cycling conditions specified in the following tables according to the instrument cycling parameters of the specific primers.

### Thermal Cycling

| Step | Temp (°C) | Time | Cycles |
|---|---|---|---|
| UDG digestion | 37° | 2 min | 1 |
| Initial denaturation* | 95° | ~30sec. | 1 |
| Denaturation | 95° | 5 sec. | 40 |
| Annealing and Extention | 60° | 15 sec. | |

*Some instruments require more than 30 seconds
ABI 7300 requires at least 31 seconds
ABI 7500 for at least 34 seconds.

## 4. Analyze the results

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information.

## *Instruments Compatibility – Rox Requirements*

* For certain instrument models, the addition of ROX reference dye is required to accurately determine the Ct value. Due to the small volume of ROX used, it is recommended to pre-mix the ROX with the qPCR Mix before use. Refer to the specific instrument manual for the required amount of ROX.

## Rox Requirements

| Platform | ROX dye | Software / set-up |
|---|---|---|
| Bio-Rad CFX series | Not required | No ROX channel / no normalization |
| Roche LightCycler 480 | Not required | No ROX normalization |
| ABI QuantStudio 3/5 | Not required | Reference dye = None |
| ABI 7500 / 7500 Fast | Not required | Reference dye= None (Low Rox optional) |
| ABI 7300/ ABI 7900HT | Recommended | Add Low / High ROX per instrument manual |
| Other Real Time Instruments | See instruments manual | Add Rox only if normalization is required |

## ROX Requirements (trouble shooting)

**ROX reference dye** is not required on instruments that support no-ROX operation: Bio-Rad CFX, LightCycler 480, and ABI QuantStudio 3/5 and ABI7500 Fast.

**Important notes**:

Our **FS-T-77241 does not contain ROX,** on the above platforms before use, to prevent baseline errors, we recommend to change the passive reference setting in your qPCR software to**:**

1) **"None"** since no passive reference dye is included
2) **or disable "ROX normalization"** in the software

You may start running the standard **reference-dye-free** workflow; the assay will run and quantify correctly.

The addition of ROX is recommended for:

**I)** Instruments that rely on ROX for normalization, such the legacy ABI 7300 / 7900HT platforms,

**(II)** Low-copy or high-precision quantification, where maximum correction of well-to-well fluorescence and thermal variation is desired

Since the volume of ROX used is very small, pre-mix ROX into the qPCR Mix before dispensing. Refer to your instrument manual for the required ROX concentration.
ROX **is not required** on no-ROX instruments: (QuantStudio 3/5, ABI-7500 Fast, Bio-Rad CFX, LightCycler 480);
ROX **is required** on ROX-dependent instruments and for low-copy or high-precision applications.

***Enhanced Benchtop reduced amplification time :***

In a triplex probe assay (FAM / ROX / CY5) 4 replicates, Human gDNA, (20 μl reactions) **FS-T-77241** amplified earlier than a leading commercial probe qPCR mix on all three channels — on average about 1 Ct, with the largest advantage (1.77 Ct) on the most competition-stressed ROX channel. Replicate CV < 2%.

**For Research Use Only**

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