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**Cat. No.** FYT105-100P FYT105-400P FYT106-100P FYT106-400P



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# EZtime Real-Time PCR Premix (2X, For TaqMan® Probe) (2X, For TaqMan® Probe, ROX)

# Description

EZtime™ Real-Time PCR Premix with TaqMan<sup>®</sup> Probe is a ready-to-use, 2X concentrated premix reagent that includes Holstart Taq, TaqMan<sup>®</sup> Probe, ROX, optimized reation buffer and AIPFs for running real-time quantitive PCR(qPCR) and 2-step aRT-PCR. This premix can be used for detection of gene and quantification of gene expression with high sensitivity, good specificity, wide dynamic range and reproducibility.

Cat. No.	Product	Volume	Package
FYT105-100P	Eztime™ Real-Time PCR Premix	1.25 ml	100 rxns
FYT105-400P	(2X, For TaqMan® Probe)	5 ml	400 rxns
FYT106-100P	Eztime™ Real-Time PCR Premix	1.25 ml	100 rxns
FYT106-400P (2X, For TaqMan® Probe, ROX)	5 ml	400 rxns	

### Contents

- EZtime<sup>™</sup> Real-Time PCR Premix (2X, TaqMan<sup>®</sup> Probe, FYT106 with ROX)
- Protocol
- Hotstart Tag DNA Polymerase
- TaqMan<sup>®</sup> Probe Real-Time PCR Buffer
- dNTP mix including dATP, dCTP, dGTP, dTTP, 5 mM MgCl<sub>2</sub>

#### Storage

-20°C, avoid repeated freezing and thawing, protected from light.

### Procedure

## A. Preparation of PCR Master Mix

- 1. Thawing all reagents completely and vortex well.
- 2. Prepare a master mix according to Table 1

## Table 1. Reaction Components for real-time PCR master mixture

Component	Volume/ reaction	Final conc.
Template DNA	2 µl	n/a
Eztime™ Real-Time PCR Premix	12.5 µl	1X
Forward Primer (10µM)	0.75 µl	0.3~0.6 µM
Reversed Primer (10µM)	0.75 µl	0.3~0.6 µM
ddH <sub>2</sub> O	9 µl	
Total	25 µl	

3. Mix the master mix thoroughly by pipetting up and down. 4. Dispense 23 ul of master mix into PCR tubes or plates.

5. Add 2 µl of the DNA or cDNA; mix carefully by pipetting up and down.

# B. Performing Real-time PCR

1. Program your instrument according to **Table 2**. Users can choose either running **(A)** 2-step real-time PCR or a traditional **(B)** 3-step real-time PCR.

# Table 2. Thermal cycling conditions.







\* X: optimal annealing temperature is depending on user's primer sequences.

2. Place the PCR tubes or PCR plates in the thermal cycler and start the cycling program.

## Applications

- Quantitative real-time PCR
- Quantitative 2-step RT-PCR
- Quick and accurate detection and quantification of target gene through real-time PCR

# Note

For research use only. Not for use in diagnostic or the rapeutic procedures.

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**Cat. No.** FYT103-100P FYT103-400P FYT104-100P FYT104-400P

# EZtime Real-Time PCR Premix (2X, For SYBR® Green) (2X, For SYBR® Green, ROX)

### Description

EZTime<sup>™</sup> Real-Time PCR Premix with SYBR® Green is a ready-to-use, 2X concentrated premix reagent that includes Hotstart Taq, SYBR® Green I, ROX, optimized reaction buffer and AURPs for running real-time quantita -tive PCR (qPCR) and 2-step aRT-PCR. This premix can be used for detec -tion of gene and quantification of gene expression with high sensitivity, wide dynamic range and reproducibility.

Cat. No.	Product	Volume	Package
FYT103-100P	Eztime™ Rea⊦Time PCR Premix	1.25 ml	100 rxns
FYT103-400P	(2X, For SYBR <sup>®</sup> Green)	5 ml	400 rxns
FYT104-100P	Eztime™ Real-Time PCR Premix	1.25 ml	100 rxns
FYT104-400P	(2X, For SYBR® Green, ROX)	5 ml	400 rxns

#### Contents

- EZtime<sup>™</sup> Real-Time PCR Premix (2X, SYBR<sup>®</sup> Green, FYT104 with ROX)
- Protocol
- Hotstart Tag DNA Polymerase
- SYBR\* Green Real-Time PCR Buffer
- dNTP mix including dATP, dCTP, dGTP, dTTP, 5 mM MgCl<sub>2</sub>

#### Storage

-20°C, avoid repeated freezing and thawing, protected from light.

### Procedure

## A. Preparation of PCR Master Mix

- 1. Thawing all reagents completely and vortex well.
- 2. Prepare a master mix according to Table 1

## Table 1. Reaction Components for real-time PCR master mixture

Component	Volume/ reaction	Final conc.
Template DNA	2 µl	n/a
Eztime™ Real-Time PCR Premix	12.5 µl	1X
Forward Primer (10 µM)	0.75 µl	0.3~0.6 µM
Reversed Primer (10 µM)	0.75 µl	0.3~0.6 µM
ddH <sub>2</sub> O	9 µl	
Total	25 µl	

Mix the master mix thoroughly by pipetting up and down.
Dispense 23 µl of master mix into PCR tubes or plates.
Add 2 µl of the DNA or cDNA. Mix carefully by pipetting up and down.

# B. Performing Real-time PCR

1. Program your instrument according to **Table 2**. Users can choose either running **(A)** 2-step real-time PCR or a traditional **(B)** 3-step real-time PCR.

# Table 2. Thermal cycling conditions.



\* 2-step program is used only for the length of target gene smaller than 300 bp.



\* X: optimal annealing temperature is depending on user's primer sequences.

- 2. Place the PCR tubes or PCR plates in the thermal cycle and start the cycling program.
- 3. Perform a melting curve analysis of the PCR product.

## Applications

- Quantitative real-time PCR
- Quantitative 2-step RT-PCR
- Quick and accurate detection and quantification of target gene through real-time PCR

## Note

For research use only. Not for use in diagnostic or the rapeutic procedures.