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TaqMan® Multiplex Master Mix

Cat. nos. 4461881, 4461882, 4461884, 4484262, 4484263, 4486295

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Component	Catalog No./Quantity						Storage
TaqMan® Multiplex Master Mix	4461881	4461882	4461884	4484262	4484263	4486295	Store at 2°C to 8°C.
	1 × 1 mL	1 × 5 mL	2 × 5 mL	5 × 5 mL	10 × 5 mL	1 × 50 mL	

This quick reference provides simplified instructions for using the TaqMan® Multiplex Master Mix reagent for performing gene expression and genotyping assays. For detailed instructions and ordering information for additional products, refer to the TaqMan® Multiplex PCR Optimization User Guide available at www.lifetechnologies.com/manuals.

Performing Real-Time PCR Assays

General Guidelines

- Mix the TaqMan® Multiplex Master Mix thoroughly before use by swirling the bottle.
- Thaw frozen samples and TaqMan® Assay mixes. Resuspend by vortexing, and centrifuge briefly.
- If preparing a master mix, scale all components except the template according to the number of reactions to be performed. Include an additional 10% of the master mix volume to account for variations in pipetting.

CHEMICAL HAZARD. TaqMan® Multiplex Master Mix (2X) may cause eye, skin and respiratory tract irritation. Read the Safety Data Sheet (SDS), and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. For safety and biohazard guidelines, refer to the Safety section in the TaqMan® Multiplex PCR Optimization User Guide.

Prepare the PCR Reactions

1. Prepare reaction components in a 1.5-mL microcentrifuge tube according to the following table:

Component	Gene Expression Analysis		Genotyping Analysis	
	384-Well Plate (10 µL/well)	96-Well Plate (20 µL/well)*	384-Well Plate (10 µL/well)	96-Well Plate (20 µL/well)*
TaqMan® Multiplex Master Mix (2X)	5 µL	10 µL	5 µL	10 µL
TaqMan® Assay Mix, FAM™ (20X)†	0.5 µL	1 µL	—	—
TaqMan® Assay Mix, VIC® (20X)†	0.5 µL	1 µL	—	—
TaqMan® Assay Mix, ABY® (20X)‡	0.5 µL	1 µL	—	—
TaqMan® Assay Mix, JUN® (20X)‡	0.5 µL	1 µL	—	—
TaqMan® Assay Mix, FAM™/VIC® (40X)†	—	—	0.25 µL	0.5 µL
TaqMan® Assay Mix, ABY®/JUN® (40X)†	—	—	0.25 µL	0.5 µL
Template	1–10 ng of cDNA		1–10 ng of gDNA	
RNase-free water	To total volume		To total volume	
Total reaction volume	10 µL	20 µL	10 µL	20 µL

* Fast protocol.
† Pre-formulated assay containing probes and primers.
‡ User formulated assay containing probes and primers.

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Prepare the PCR Reactions, continued

2. Mix the components thoroughly, then centrifuge briefly to spin down the contents and eliminate any air bubbles.
3. Transfer the appropriate volume of each reaction to each well of an optical plate.
4. Seal the plate with an optical adhesive cover, then centrifuge the plate briefly to spin down the contents and eliminate any air bubbles.

Run the PCR Reaction Plate

Run the plate on a Life Technologies real-time quantitative PCR instrument. See the appropriate instrument user guide for help with programming the thermal-cycling conditions or with running the plate. To run the plate:

1. Place the reaction plate in the instrument.
2. Set the thermal cycling conditions using the default fast PCR thermal-cycling conditions specified in the following tables:

ViiA™ 7 and QuantStudio™ Real-Time PCR Systems			
Step	**Temperature**	**Duration**	**Cycles**
AmpliTaq® Fast DNA Polymerase, UP Activation	95°C	20 sec	Hold
Denature*	95°C	1 sec	40
Anneal/Extend	60°C	20 sec	

7500/7500 Fast Real-Time PCR Systems			
Step	**Temperature**	**Duration**	**Cycles**
AmpliTaq® Fast DNA Polymerase, UP Activation	95°C	20 sec	Hold
Denature*	95°C	3 sec	40
Anneal/Extend	60°C	30 sec	

* Denature time can be increased if the amplicon size is long.

3. Set the reaction volume appropriate for the type of plate being used for your PCR reaction.
4. Start the run.

Analyze Your Results

- View the amplification plots.
- Adjust the baseline and threshold values to determine the threshold cycles (C_T) for the amplification curves.
- Use the standard curve method or the relative quantification ($\Delta\Delta C_T$) method to analyze results.

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