TaqMan® Universal Master Mix II

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan® Universal Master Mix II Protocol* (PN 4428173). For all chemicals, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

TaqMan® Universal Master Mix II is a convenient mix of components (except primers, probes, template, and water) necessary to perform real-time polymerase chain reaction (PCR). The following protocol describes how to perform real-time PCR quantitation using TaqMan® Universal Master Mix II and TaqMan® assays. For information on preparation methods for other applications, refer to the *TaqMan® Universal Master Mix II Protocol* (PN 4428173).

1 Thaw and mix the reagents

a. If necessary, thaw the TaqMan® assay on ice.

b. When the reagents are thawed completely, gently vortex and briefly centrifuge the tubes to resuspend the reagents. Mix the TaqMan® Universal Master Mix II by gently swirling the bottle.

2 Calculate the number of reactions

Calculate the number of reactions that you need for each assay based on the number of samples and the reaction formation that you are running.

Note: Applied Biosystems recommends performing four replicates of each reaction.

3 Prepare the PCR reaction mix

a. For each sample, pipette the following to a sterile 1.5- or 2-mL microcentrifuge tube. Multiply the volumes as needed based on the number of replicates that you are running.

Note: Applied Biosystems recommends preparing 110% of the required volume to account for pipetting error.

PCR reaction mix component	Volume per reaction (µL)		Final conc.
	50-µL rxns.	20-µL rxns.	
TaqMan® Universal Master Mix II, with/no UNG, 2×	25.0	10.0	1×
TaqMan® Assay, 20×	2.5	1.0	1×
cDNA or DNA template + RNase-free water‡	22.5	9.0	1 to 100 ng
Total Volume	**50.0**	**20.0**	**—**

‡ To each reaction, add 1 to 100 ng of cDNA or DNA template diluted to the correct volume using RNase-free water.

b. Cap each tube and invert several times to mix.

c. Centrifuge the tubes briefly.

4 Prepare the reaction plate

a. For each sample, transfer 50 µL of PCR reaction mix to the associated wells of the 96-well reaction plate.

Note: If you are using a real-time PCR system with a 384-well sample block, scale the reaction volume accordingly to 20-µL.

b. Seal the plate using the appropriate cover.

c. Centrifuge the plate briefly.

d. Load the plate into the real-time PCR system.

IMPORTANT! Run the reaction plate within 2 hours of completing the reaction setup. Otherwise, refrigerate the plate until you can load it into the instrument.

5 Run the plate

a. Create a plate document or experiment for the run using the parameters in the following table.

System	UNG incubation‡	Polymerase activation	PCR	
	Hold	Hold	Cycle (40 cycles)	
			Denature	Anneal/extend
Temperature (°C)	50	95	95	60
Time (mm:ss)	2:00	10:00	00:15	1:00
Reaction volume (µL)	50 or 20			

‡ Required for optimal Uracil-N-Glycosylase (UNG) activity; not needed when UNG is not present in the reaction.

b. Run the plate.

6 Analyze the data

To analyze the data:

- View the amplification plots for the entire plate.
- Set the baseline and threshold values.
- Use the relative standard curve or the comparative C_T method to analyze your data.

The details of the data analysis depend on the real-time PCR instrument used to run the plate. Refer to the appropriate instrument user documentation for instructions on how to analyze your data.

Ordering Information

The TaqMan® Universal Master Mix II, with or no UNG, is supplied in a 2X concentration and is available in the quantities listed in the following table. To order TaqMan® Universal Master Mix II, or associated TaqMan® reagents and assays, go to: **www.appliedbiosystems.com**

Product	Item	Volume	50-µL reactions	Part number
TaqMan® Universal Master Mix II, with UNG Includes: • AmpliTaq Gold® DNA Polymerase, UP (UltraPure) • Uracil-N-Glycosylase (UNG) • dNTPs with dUTP • Passive Reference 1 • Optimized buffer components	Mini-Pack	1 × 1-mL tube	40	4440042
	1-Pack	1 × 5-mL bottle	200	4440038
	2-Pack	2 × 5-mL bottles	400	4440044
	5-Pack	5 × 5-mL bottles	1000	4440045
	10-Pack	2 × 5-mL bottles	2000	4440046
	Bulk-Pack	1 × 50-mL bottle	2000	4440039
TaqMan® Universal Master Mix II, no UNG Includes: • AmpliTaq Gold® DNA Polymerase, UP (UltraPure) • dNTPs with dUTP • Passive Reference 1 • Optimized buffer components	Mini-Pack	1 × 1-mL tube	40	4440043
	1-Pack	1 × 5-mL bottle	200	4440040
	2-Pack	2 × 5-mL bottles	400	4440047
	5-Pack	5 × 5-mL bottles	1000	4440048
	10-Pack	2 × 5-mL bottles	2000	4440049
	Bulk-Pack	1 × 50-mL bottle	2000	4440041

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER: PLEASE REFER TO THE TAQMAN® UNIVERSAL MASTER MIX II PROTOCOL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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