

SYBR® Select Master Mix

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This quick reference card provides simplified instructions for using the SYBR® Select Master Mix reagent for performing real-time PCR assays. For detailed instructions and ordering information for additional products, refer to the SYBR® Select Master Mix User Guide available at www.lifetechnologies.com/manuals.

Performing Real-Time PCR Assays

General Guidelines

- Prepare single-stranded cDNA from total RNA or mRNA samples. For optimal performance, use up to 100 ng of cDNA for 10 µL or 20 µL reactions.
- Mix the SYBR® Select Master Mix thoroughly before use by swirling the bottle.
- For best results, it is recommended to perform four replicates of each reaction.
- Master mixes can be prepared depending upon your experimental requirements. Scale the components to be included in the master mix according to the number of reactions to be performed. Include an additional 10% of the master mix volume to account for variations in pipetting.
- If using smaller reaction volumes, scale all components of the reaction mix proportionally. Reaction volumes <10 µL are not recommended.

CHEMICAL HAZARD. SYBR® Select 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 SYBR® Select Master Mix Protocol.

Prepare the PCR Reactions

1. Prepare the appropriate number of reactions according to the volumes in the following table:

Component	384-Well Plate (10 µL/well)	48-Well or 96-Well Plate (20 µL/well)
SYBR® Select Master Mix (2X)	5 µL	10 µL
Forward and reverse primers‡	Variable	Variable
cDNA template + RNase-free water	Variable	Variable
Total reaction volume	10 µL	20 µL

‡ For optimal performance in Standard mode, use a range from 150–400 nM for each primer. If using StepOne® or StepOnePlus® Real-Time PCR Systems, use ≤200 nM of each primer. For optimal performance in in Fast mode (384-well plates), increase primer concentration toward the higher end of the recommended range (e.g. 400 nM). If using StepOne® or StepOnePlus® Real-Time PCR Systems in Fast mode, use 300–400 nM of each primer.

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.

Note: PCR can be performed on the reaction plate at any time up to 72 hours after completing the reaction setup when kept at room temperature, and protected from exposure to direct light.

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 PCR thermal-cycling conditions specified in the following tables according to the melting temperature of your primers:

Standard Cycling Mode (Primer T _m ≥60°C)			
Step	Temperature	Duration	Cycles
UDG Activation	50°C	2 min	Hold
AmpliTaq® Fast DNA Polymerase, UP Activation	95°C	2 min	Hold
Denature	95°C	15 sec	40
Anneal/Extend	60°C	1 min	

Fast Cycling Mode (Primer T _m ≥60°C)			
Step	Temperature	Duration	Cycles
UDG Activation	50°C	2 min	Hold
AmpliTaq® Fast DNA Polymerase, UP Activation	95°C	2 min	Hold
Denature	95°C	1*or 3** sec	40
Anneal/Extend	60°C	30 sec	

* Denature for 1 second when using ViiA™ 7, or QuantStudio™ 12K Real-Time PCR Systems.

** Denature for 3 seconds when using 7500 Fast, StepOne®, or StepOnePlus® Real-Time PCR Systems.

3. Set the instrument to perform a default dissociation step. **Note:** A dissociation curve can be performed up to 72 hours after the real-time PCR run if the plate is stored in the dark, or up to 24 hours later if the plate is stored exposed to light.
4. Set the reaction volume appropriate for the type of plate being used for your PCR reaction.
5. 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 (ΔΔC_T) method to analyze results.
- When performing melt curves with StepOne®, or StepOnePlus® instruments, deselect the passive reference (see manual for details).

NOTICE TO PURCHASER: PLEASE REFER TO THE SYBR® SELECT MASTER MIX USER GUIDE FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION. LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

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