# Guidelines for comparing SYBR Green master mixes

Take the master mix challenge—compare your current SYBR™ Green master mix to Applied Biosystems™ PowerTrack™ SYBR™ Green Master Mix using this simple protocol. In our own experiments, we found that PowerTrack SYBR Green Master Mix outperformed other master mixes when comparing specificity, PCR efficiency, dynamic range, and precision over multiple targets.

This protocol is for the comparison of PowerTrack SYBR Green Master Mix to one other master mix, using 20 µL reactions in triplicate. Adjust volumes as needed.

**General guidelines**

- Use the manufacturer's recommended primer and target DNA concentrations.
- Use the manufacturer's recommended thermal cycling parameters.
- Run standard curves to compare PCR efficiency, sensitivity, dynamic range, and precision.
- Run reactions in triplicate to measure reproducibility.
- Perform a melt curve to analyze specificity.

## 1. Prepare reactions.

a. Prepare a 10-fold dilution series of the template.

| Dilution | Template | Nuclease-free water |
|---|---|---|
| Undiluted (20 ng/µL) | 50 µL of undiluted | — |
| $10^{-1}$ | 5 µL of undiluted | 45 µL |
| $10^{-2}$ | 5 µL of $10^{-1}$ | 45 µL |
| $10^{-3}$ | 5 µL of $10^{-2}$ | 45 µL |
| $10^{-4}$ | 5 µL of $10^{-3}$ | 45 µL |
| $10^{-5}$ | 5 µL of $10^{-4}$ | 45 µL |
| $10^{-6}$ | 5 µL of $10^{-5}$ | 45 µL |

b. Add 5 µL of Yellow Sample Buffer to the $10^{-6}$ dilution, and 4.5 µL to all of the other dilutions.

c. For each master mix to be tested, prepare enough master mix for 26 reactions (20 µL each), according to the table below. Mix thoroughly.

| Component | PowerTrack SYBR Green Master Mix | Other master mix |
|---|---|---|
| qPCR master mix, 2X | 260 µL | 260 µL |
| Forward primer, 10 µM | 26 µL* | See manufacturer's recommendation |
| Reverse primer, 10 µM | 26 µL* | |
| Nuclease-free water | 65 µL (to bring to 377 µL) | Bring volume to 377 µL |

* Amount shown is for a final primer concentration of 500 nM. The recommended primer concentration range for PowerTrack SYBR Green Master Mix is 300–800 nM.

d. Pipet 14.5 µL of the PowerTrack SYBR Green Master Mix prepared in step c to wells of a 96-well optical plate, according to the plate map below.

e. Pipet 5.5 µL of each template dilution from step a into the wells used in step d according to the plate map below. For no-template control (NTC) reactions, use 5.5 µL of nuclease-free water.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | 1x | 1x | 1x | | | | | |
| B | | | | | $10^{-1}$x | $10^{-1}$x | $10^{-1}$x | | | | | |
| C | | | | | $10^{-2}$x | $10^{-2}$x | $10^{-2}$x | | | | | |
| D | | | | | $10^{-3}$x | $10^{-3}$x | $10^{-3}$x | | | | | |
| E | | | | | $10^{-4}$x | $10^{-4}$x | $10^{-4}$x | | | | | |
| F | | | | | $10^{-5}$x | $10^{-5}$x | $10^{-5}$x | | | | | |
| G | | | | | $10^{-6}$x | $10^{-6}$x | $10^{-6}$x | | | | | |
| H | | | | | NTC | NTC | NTC | | | | | |

f. Repeat steps c–e for the other master mix to be compared, using a new plate. **Note:** Reactions for both master mixes can be loaded on the same plate if the thermal protocols are identical.

g. Mix the components thoroughly. Seal the plates, and briefly centrifuge to remove any bubbles.

## 2. Run the real-time PCR.

a. Place the 96-well plate in the instrument.

b. Set the thermal cycling conditions. For PowerTrack SYBR Green Master Mix, use one of the following:

c. Run the plate.

**Standard cycling conditions (for most instruments, and primer $T_m$ ≥60°C)***

| Step | Temperature | Duration | Cycles |
|---|---|---|---|
| Polymerase activation | 95°C | 2 min | Hold |
| Denaturation | 95°C | 15 sec | 40 |
| Annealing/extension | 60°C | 1 min | |

* For primers with $T_m$ <60°C, please refer to the PowerTrack SYBR Green Master Mix instruction manual.

**Fast cycling conditions (for Applied Biosystems™ ViiA™ 7, QuantStudio™, 7500 Fast, StepOne™, and StepOnePlus™ instruments, and primer $T_m$ ≥60°C)***

| Step | Temperature | Duration | Cycles |
|---|---|---|---|
| Polymerase activation | 95°C | 2 min | Hold |
| Denaturation | 95°C | 5 sec | 40 |
| Annealing/extension | 60°C | 30 sec | |

* For primers with $T_m$ <60°C, please refer to the PowerTrack SYBR Green Master Mix instruction manual.

**For Applied Biosystems instruments, use the following settings:**

| | |
|---|---|
| **Experiment type** | Standard curve |
| **Reagent** | SYBR Green |
| **Reporter** | SYBR Green dye |
| **Quencher** | None |
| **Passive reference dye** | ROX |
| **Ramp speed** | Standard or Fast (see thermal cycler profiles above) |

See instrument instruction manual for more information or if using a different instrument.

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| | |
|---|---|
| **Experiment type** | Melt curve |
| **Reagent** | SYBR Green |
| **Reporter** | SYBR Green dye |
| **Quencher** | None |
| **Passive reference dye** | ROX |
| **Ramp speed** | Standard |
| **Melt curve ramp increment** | Continuous (default) |

### 3. Run the melt curve.

For Applied Biosystems instruments, use settings and conditions as noted in these two tables:

| Stage | Step | Ramp rate | Temperature | TIme |
|---|---|---|---|---|
| Melt curve | Step 1 | 1.6°C/sec | 95°C | 15 sec |
| | Step 2 | 1.6°C/sec | 60°C | 1 min |
| | Step 3 (dissociation) | 0.05°C/sec | 95°C | 15 sec |

### 4. Repeat steps 2–3 for the other master mix.

Use the other manufacturer's recommended thermal cycling profile.

### 5. Analyze the data.

On most qPCR systems, the data can be analyzed for the following parameters by the qPCR system software.

a. **Slope and amplification efficiency**
The amplification efficiency is calculated using the slope of the regression line in the standard curve. A slope of –3.3 indicates optimal, 100% PCR amplification efficiency. Reactions with efficiencies of 100% ± 10% over a broad dynamic range are needed to reliably use the $\Delta\Delta C_t$ method for gene expression analysis.

b. **$R^2$ values (correlation coefficient)**
The $R^2$ value is a measure of the closeness of fit between the regression line and the individual $C_t$ data points of the standard curve reactions. An $R^2$ value of 1.00 indicates a perfect fit between the regression line and the data points; a value of >0.99 is desirable.

c. **Standard deviation (precision)**
For each dilution, the standard deviation of the $C_t$ values is calculated. To be able to quantify a 2-fold dilution with ≥95% confidence, the standard deviation of a dilution should be ≤0.250.

d. **Melt curve (specificity)**
A melt curve is viewed using a derivative reporter plot (derivative reporter (–Rn) vs. temperature). A single peak indicates specific amplification, whereas multiple peaks or shoulders indicate nonspecific amplification or primer-dimer formation.

**Caution:** Don't be fooled by lower $C_t$ values when comparing master mixes. The only way to compare the sensitivity and performance of two master mixes is to perform a dilution series and examine the amplification efficiency, linearity, and precision across that range.

Find out more at **thermofisher.com/sybr**

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