Compare your SYBR™ green mix to Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix

Assess analytical performance for your gene expression workflow using a side-by-side serial dilution experiment

What to evaluate

Efficiency
Calculated from the standard curve slope

Linearity
R^2 value across the dilution series

Precision
Replicate C_t consistency at each dilution

Analytical specificity
Single peak in melt curve analysis

General guidelines

- ☑ Use the manufacturer's recommended primer and template DNA concentrations
- ☑ Use the manufacturer's recommended thermal cycling parameters
- ☑ Keep plate format, reaction volume, and analysis settings consistent where possible
- ☑ Include a no-template control for each target
- ☑ Run reactions in triplicate to measure precision
- ☑ Perform a melt curve to assess analytical specificity

Step 1: prepare a dilution series

Prepare a 10-fold dilution series from a single complementary DNA (cDNA) sample source. For comparison, the same dilution point can be used for all assays. This example provides enough volume to run one dilution point in triplicate for each real-time PCR (qPCR) assay mix, for up to four assay mixes (see diagram on page 2).

Dilution	Template	Nuclease-free water
Undiluted	100 µL cDNA template	-
10^{-1}	10 µL of undiluted	90 µL
10^{-2}	10 µL of 10^{-1}	90 µL
10^{-3}	10 µL of 10^{-2}	90 µL
10^{-4}	10 µL of 10^{-3}	90 µL
10^{-5}	10 µL of 10^{-4}	90 µL
10^{-6}	10 µL of 10^{-5}	90 µL

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Step 2: set up the reactions

The reaction setup below uses target-specific forward and reverse primers in a 96-well plate with a 20 µL reaction volume. For other primer formats, follow the manufacturer's recommended setup.

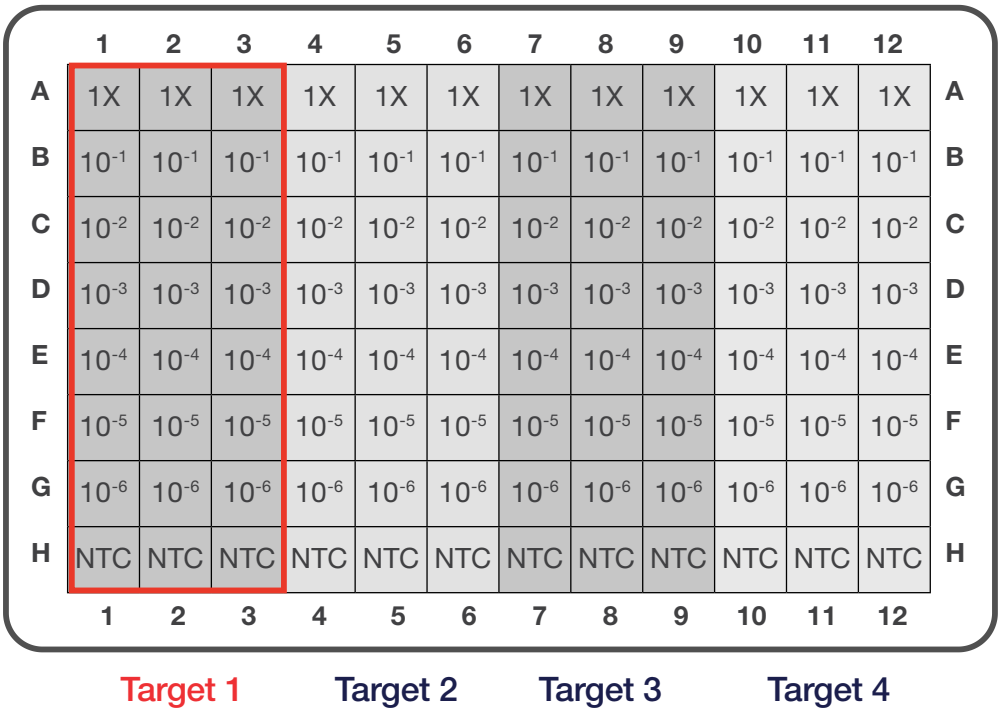
1. For the first assay, use the table below to prepare enough PowerUp SYBR Green Master Mix reaction mix for 26 reactions (20 µL each). Mix thoroughly.

Component	Volume for 26 reactions (24 reactions + overage)
PowerUp SYBR Green Master Mix (2X)	260 µL
Forward Primer	26 µL
Reverse Primer	26 µL
Nuclease-free water	78 µL
Total volume	**390 µL**

Note: *Example shown for 500 nM final primer concentration. Recommended range: 300–800 nM.

2. Pipette 15 µL of the reaction mix containing PowerUp SYBR Green Master Mix into the appropriate wells of a 96-well optical plate, according to the plate map below.

3. Add 5 µL of each template dilution from step 1 into the appropriate wells, according to the plate map below. For no-template control (NTC) reactions, use 5 µL of nuclease-free water.



4. Repeat steps 1–3 for any additional qPCR mixes, up to four assays per plate.

5. Seal the plate, then briefly centrifuge.

6. Start your qPCR run.

7. If comparing performance to another master mix, repeat steps 1–6 using the other master mix and a new plate.

Step 3: run qPCR

Run each plate according to the cycling conditions provided in the reagent user documentation. Include a melt curve analysis following amplification.

Fast cycling conditions for PowerUp SYBR Green Master Mix

Step	Temperature	Time	Cycles
UDG activation	50°C	2 min	Hold
Polymerase activation	95°C	2 min	Hold
Denaturation	95°C	1 sec	40x
Anneal/extend	60°C	30 sec	

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Will these cycling conditions work with my qPCR instrument?

The PowerUp SYBR Green Master Mix user guide provides these cycling conditions as a starting point for most instruments, including Bio-Rad™ CFX Opus 96 and Roche™ LightCycler™ PRO systems.

Cycling conditions can vary by assay and instrument, so refer to the appropriate user documentation as needed.

Will the Applied Biosystems ™ ROX™ dye in PowerUp SYBR Green Master Mix affect the accuracy of my results?

PowerUp SYBR Green Master Mix contains ROX dye as a passive reference. Some systems, such as Bio-Rad and Roche qPCR systems, do not use ROX dye for passive reference normalization.

If you are comparing a master mix without ROX dye to one containing ROX dye, you may notice differences in C_t values or baseline signal. Evaluate efficiency, precision, linearity, and sensitivity rather than relying on C_t values alone.

Refer to your instrument and analysis software documentation for dye compatibility and normalization settings.

Step 4: analyze the results

Review the standard curve in your analysis software to identify the slope of the standard curve, efficiency, R^2, and replicate C_t values. Refer to your software documentation for analysis instructions.

Efficiency

Review the efficiency value calculated by your analysis software from the slope of the standard curve. Values between 90–110% are generally acceptable.

Precision

Review the standard deviation (SD) of technical replicate C_t values at each dilution. Lower SD values indicate better replicate agreement, and SD ≤0.250 is generally acceptable.

Linearity

Review the R^2 value for the standard curve across the dilution series. Values closer to 1.0 indicate better linearity, and R^2 >0.99 is generally acceptable.

Analytical specificity

Review the melt curve to confirm the absence of nonspecific amplification. A single peak indicates amplification of a single product.

Don’t be misled by lower C_t values alone. Review the full dilution series and use the evaluation scorecard on the next page to compare results.

Evaluation scorecard

Use the scorecard below to compare performance for each assay across both qPCR mixes. When reviewing the dilution series, note that the usable dynamic range is defined by the portion of the dilution series where both amplification efficiency and linearity remain acceptable.

Tip: use the "Notes" column to capture observations from the dilution series, including the range where efficiency and linearity remain acceptable, replicate variability, or any setup or analysis issues.

Target		Efficiency (%)	Linearity (R^2)	Precision (SD)	Analytical specificity (Melt Curve)	Notes
1	Current mix					
	PowerUp SYBR Green Master Mix					
2	Current mix					
	PowerUp SYBR Green Master Mix					
3	Current mix					
	PowerUp SYBR Green Master Mix					
4	Current mix					
	PowerUp SYBR Green Master Mix					

Finished your evaluation? Choose your next step

Complete the evaluation survey

Tell us how the master mix performed and request specialist follow-up for quote support (optional).

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PowerUp SYBR Green Master Mix

Cat. Nos.: A25741, A25779, A25780, A25918, A25742, A25776, A25777, A25778, A25743

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