SsoFast™ EvaGreen® Control Assay Instructions

About the Assay

The SsoFast EvaGreen control assay is a pre-designed kit for training new users on real-time PCR or verifying the performance of your real-time PCR system.

- Demonstrates a broad dynamic range from 10^2 to 10^8 copies
- Validated for use on the CFX96™, CFX384™, MiniOpticon™, and MyiQ™2 real-time PCR detection systems
- Includes instructions for setting up a dilution series to create a standard curve

Storage and Stability

Store at –20°C. After thawing, remaining stock reagents may be stored at 4°C for up to 6 months. Avoid repeated freeze-thaw cycles. Unused master mix and template DNA dilutions should be discarded.

Kit Contents

The SsoFast EvaGreen control assay includes enough reagents to run the dilution series three times (Table 1).

Table 1. SsoFast EvaGreen control assay kit contents.

Component	Concentration	Volume
SsoFast EvaGreen supermix	2x	Two 1 ml tubes
Control assay template	10^8 copies/µl	70 µl
Control assay primer mix	100x (50 µM each primer)	60 µl
Nuclease-free water	NA	Two 1.5 ml tubes

Additional Materials Required

- Sterile, nuclease-free 1.5 ml tubes for dilutions
- Sterile, nuclease-free Tris-EDTA (TE) buffer for dilutions (10 mM Tris, 0.1 mM EDTA, pH 8.0 is recommended)*
- Compatible 48-well, 96-well, or 384-well PCR plate and sealer, as recommended for your real-time PCR detection system
- Pipets and filtered pipet tips to prevent cross-contamination
- UV hood to prevent cross-contamination (optional)

* If TE buffer is not available, use sterile, nuclease-free water.

IF THIS ASSAY WAS SENT TO YOU BY TECHNICAL SUPPORT, PLEASE FOLLOW THE INSTRUCTIONS EMAILED TO YOU.

Preparing the Template DNA Dilutions

1. Pipet 90 µl of TE buffer into seven 1.5 ml tubes labeled 2–8.
2. Prepare the dilution series according to the directions in Table 2. After adding the template DNA to each tube, mix by repeatedly pipeting up and down ten times. Do not vortex the DNA dilutions.
3. Continue with the 96-Well or 384-Well Plate Standard Curve Setup section appropriate for your instrument.

Table 2. Preparation of dilution series.

Tube	TE Buffer, µl	Template DNA	Final [DNA], copies/µl
1	—	Control assay template stock	10^8
2	90	10 µl from Tube 1	10^7
3	90	10 µl from Tube 2	10^6
4	90	10 µl from Tube 3	10^5
5	90	10 µl from Tube 4	10^4
6	90	10 µl from Tube 5	10^3
7	90	10 µl from Tube 6	10^2
8	90	—	No template control (NTC)

Note: Tube 1 does not require dilution. Tube 1 is the control assay template stock that comes with the kit.

96-Well Plate Standard Curve Setup

Preparing the Master Mix

1. Thaw all the components. Gentle rocking will help dissolve the SsoFast EvaGreen supermix. Take care not to generate bubbles or foam.
2. Add the components listed in Table 3 to a 1.5 ml tube and mix thoroughly, but gently.

Table 3. Volumes of components for standard curve setup.

Component	Volume, µl
SsoFast EvaGreen supermix (2x)	650
Control assay primer mix (100x)	13
Nuclease-free water	572
Total volume of master mix	1,235

Preparing the Reactions

1. Label eight 1.5 ml tubes 1–8.
2. Pipet 133 µl of the master mix into each tube.
3. Pipet 7 µl of the corresponding template DNA dilution into each tube.
4. Mix contents thoroughly by vortexing gently.
5. Briefly centrifuge the tubes.

Pipeting the Reactions into a Plate

1. Pipet four replicates of each reaction into a 96-well plate using 20 µl per well (Figure 1). Start pipeting the lowest concentration first, ending with the highest concentration. A 100 µl electronic repeat pipeter is helpful, but is not required.
2. Completely seal the plate with a compatible sealer.
3. Centrifuge the plate to settle its contents.
4. Run the plate using the protocol specified in the Thermal Cycling Conditions section.

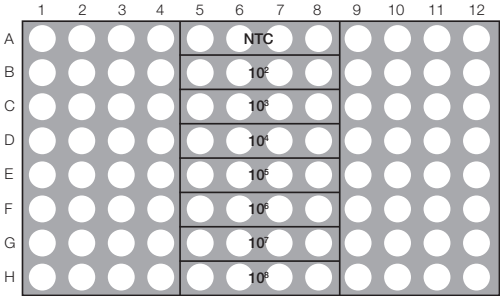

Fig. 1. 96-well plate layout — 20 µl/well.

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384-Well Plate Standard Curve Setup

Preparing the Master Mix

1. Thaw all the components. Gentle rocking will help dissolve the SsoFast EvaGreen supermix. Take care not to generate bubbles or foam.
2. Add the components listed in Table 4 to a 1.5 ml tube and mix thoroughly, but gently.

Table 4. Volumes of components for standard curve setup.

Component	Volume, µl
SsoFast EvaGreen supermix (2x)	450
Control assay primer mix (100x)	9
Nuclease-free water	351
Total volume of master mix	810

Preparing the Reactions

1. Label eight 1.5 ml tubes 1–8.
2. Pipet 90 µl of the master mix into each tube.
3. Pipet 10 µl of the corresponding template DNA dilution into each tube.
4. Mix contents thoroughly by vortexing gently.
5. Briefly centrifuge the tubes.

Pipeting the Reactions into a Plate

1. Pipet six replicates of each reaction into a 384-well plate using 10 µl per well (Figure 2). Start pipeting the lowest concentration first, ending with the highest concentration. A 100 µl electronic repeat pipeter is helpful, but is not required.
2. Completely seal the plate with a compatible sealer.
3. Centrifuge the plate to settle its contents.
4. Run the plate using the protocol specified in the Thermal Cycling Conditions section.

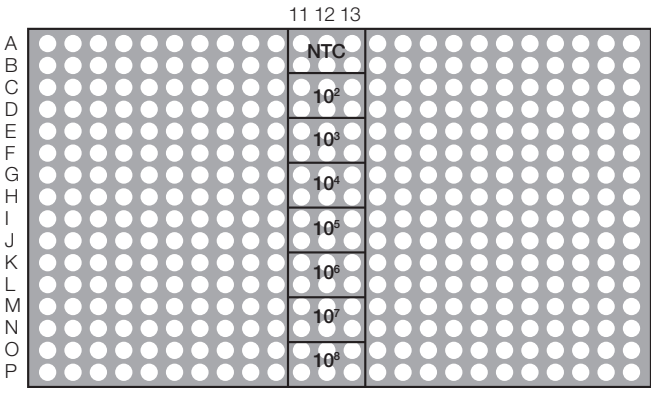

Fig. 2. 384-well plate layout — 10 µl/well.

Thermal Cycling Conditions

1. Program the real-time PCR instrument using the appropriate plate layout (Figure 1 or 2) and the appropriate thermal cycling protocol for your instrument (Table 5).
2. Place the plate in the real-time PCR instrument and run the program.

Table 5. Thermal cycling protocols for real-time PCR detection systems.

CFX96 and CFX384 Protocol	MyiQ2 Protocol	MiniOpticon Protocol
95°C for 30 sec	95°C for 30 sec	95°C for 30 sec
95°C for 1 sec, then 60°C for 5 sec (x 40 cycles)	95°C for 1 sec, then 60°C for 10 sec (x 40 cycles)	95°C for 5 sec, then 60°C for 10 sec (x 40 cycles)
Default melt curve from 60–95°C	Melt curve from 60–95°C, 30 sec hold	Melt curve from 60–95°C, 5 sec hold

Analyzing the Data

1. Analyze the data using the default automatic baseline option.
2. With the fluorescence data in log scale view, adjust the threshold such that it is in the linear range of the amplification traces and the reaction efficiency is relatively insensitive to the exact threshold setting (Figure 3).
3. Note the efficiency and R^2 value of the standard curve.

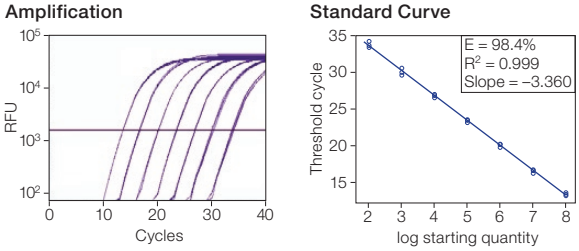

Fig. 3. Sample data for a standard curve on a CFX96 real-time PCR detection system. RFU, relative fluorescence units.

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