



Catalog #: S0140

## **FAM + HEX Calibrator Set**

For research use only

Store at 4 to 20 °C before reconstitution

Store at 4 °C after reconstitution

Do not freeze once reconstituted

Protect from light

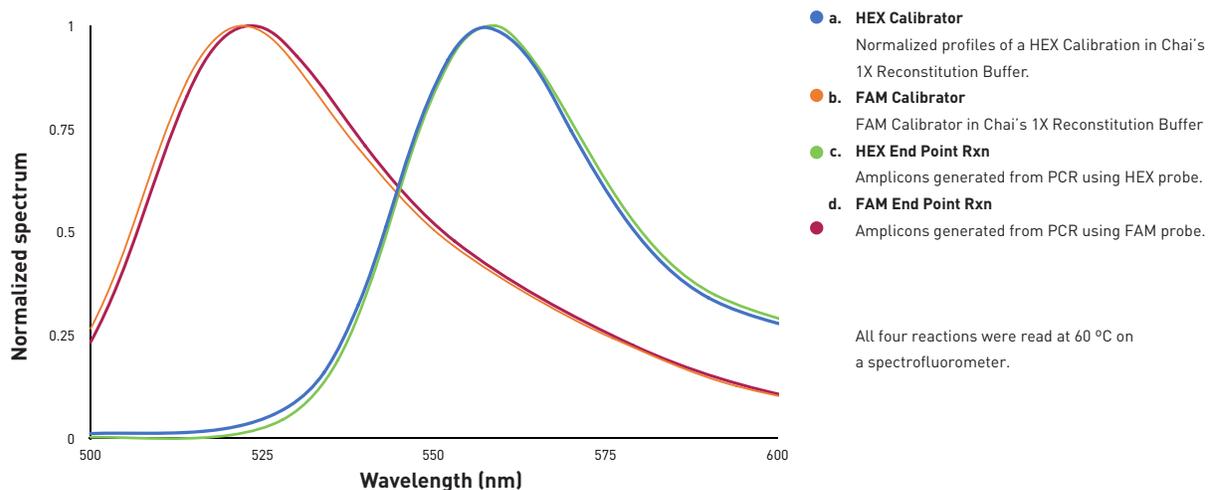
## INTRODUCTION

Chai's HEX and FAM calibrators are designed to calibrate the dual channel Open qPCR instrument. The FAM fluorophore is read in Channel 1 of the Open qPCR whereas the HEX fluorophore is read in Channel 2. The FAM + HEX Calibrator Set contains all the necessary reagents to run calibration on the Open qPCR instrument quickly and efficiently.

It is important to select dyes that are compatible and can be calibrated with the thermal cycler. The emission peaks of the FAM and HEX fluorophores are distinctly separated, thus differentiating the signals. The combination of these two dyes provides confidence that the signal detected in a particular well originates from one specific probe. However, if  $\lambda_{\text{max}}$  (absorption) and  $\lambda_{\text{max}}$  (emission) overlapping regions occur for two or more dyes, this would potentially result in a cross-talk effect. When multiplexing an assay, crosstalk occurrence will highly impact  $C_q$  values due to signal bleed-through into adjacent filter-sets.

The calibration standards in this kit are designed to mimic signals from the FAM and HEX fluorescent probes. During the calibration process, the software stores a fluorophore profile utilizing these two standard dyes as a reference point. The software then uses the stored fluorophore profile to determine how much fluorescence to expect during amplification of said fluorophore. This data is important for future experimental runs as it resolves any cross-talk activity between the two channels and correct for well-to-well variation in the instrument's optical path.

Figure 1 below illustrates comparative fluorescence spectra of the kit calibrators and probe-based endpoint reactions. Probe-based endpoint reactions are PCR reactions that contain FAM or HEX probes amplified for 40 cycles. The FAM calibrator/FAM end-point reaction and the HEX calibrator/HEX end-point reaction closely overlaps with one another. Such overlap is indicative of a good calibrator since the calibrator signal resembles the signal produced from an assay with the same fluorophore.



**Figure 1.** Normalized spectrum profiles of: a. HEX Calibrator in Chai's 1X Reconstitution Buffer. b. FAM Calibrator in Chai's 1X Reconstitution Buffer. c. Amplicons generated from PCR using HEX probe. d. Amplicons generated from PCR using FAM probe. All four reactions were read at 60 °C on a spectrofluorometer.

The FAM and HEX sequences are designed with the fluorophore covalently linked to the 5' end of a 10-mer DNA oligo. Depending on the chemistry of the probe used in a qPCR assay, during PCR, the fluorophore remains attached to the probe (i.e. Scorpion probes) or gets cleaved from the probe with few bases remaining attached to it (i.e. TaqMan probes). The FAM + HEX Calibrator Set serves as an ideal calibrator as it reflects the chemistry of both probes.

## SPECIFICATIONS

The FAM and HEX Calibrators are provided in a lyophilized format in separate tubes. The Reconstitution Buffer is provided as a third component of the calibration kit. Once the calibrators are reconstituted, they are ready for use.

Storage temperature of the lyophilized calibrators and the reconstitution buffer should be at 4 to 20 °C. Lyophilized calibrators are light sensitive. Ensure that the vials are placed in a dark location. The reconstituted calibrators are stable for one year when stored at 4 °C and protected from light.

**\*TECHNICAL NOTE:** The fluorescence spectra of the FAM and HEX calibrators depend on the buffer composition. The provided reconstitution buffer is representative of many master mixes. Alternatively, the calibrators may be reconstituted in the master mix that will be used in the qPCR reaction for optimal results.

### KIT COMPONENTS

Product Name	Calibrator Sequence	Amount/Volume	Quantity
FAM Calibrator	5-FAM-TTTTTTTTTT	0.68 nmol per vial	1
HEX Calibrator	6-HEX-TTTTTTTTTT	1.35 nmol per vial	1
Reconstitution Buffer	-	1.5 mL per vial	2

\*TECHNICAL NOTE: The final concentrations of the reconstituted FAM and HEX calibrators are at 515 nM and 1022 nM, respectively.

### WORKFLOW

The calibration process should take approximately 25 minutes to complete (excluding the reconstitution step).

1. Reconstitute the lyophilized FAM and HEX calibrators
2. Prepare sterile strip tubes, sterile water, FAM solution, HEX solution
3. Run calibration on instrument

### RECONSTITUTION BUFFER FORMULA

The table below illustrates the working concentration for each of the components in the provided Reconstitution Buffer.

Components	1X Concentration
Tris-HCl, pH 8.6	10 mM
KCl	50 mM
MgCl <sub>2</sub>	3 mM
Glycerol	5%

### **RECONSTITUTION PROCEDURES**

1. Prior to opening the calibrator tubes, use a mini centrifuge to spin down the two lyophilized HEX and FAM vials. It is important to gather all materials at the bottom of the tube.
2. Using a sterile tip, pipette 1.32 mL of the reconstitution buffer into each of the calibrator vials. Mix well by pipetting the solution up and down ten times before capping the vials. Alternatively, ensure vial caps are tightly secured before vortexing the vials for 5-10 seconds to ensure homogenous mixing.
3. Incubate the vials at room temperature for at least thirty minutes. This is necessary to ensure that the product has completely dissolved. During the thirty-minute incubation period, protect the calibrator vials from light by placing them in an opaque drawer or covering them with aluminum foil.
4. Following the thirty-minute incubation, open the vial caps and use a sterile tip to pipette up and down ten times. Alternatively, ensure vial caps are tightly secured before vortexing the vials for 5-10 seconds to ensure homogenous mixing.
5. Spin down the vials using a mini centrifuge to collect all liquid contents at the bottom of the tube. If a centrifuge is unavailable, flick the tubes in a downward motion to gather liquid contents at the bottom. The reconstituted calibration vials are now ready for use.

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