The stability of KAPA SYBR FAST Universal 2x qPCR MasterMix was assessed by qPCR of the human β-actin gene. Reactions were performed in 20 µL volumes, with 200 nM of each primer and 16 ng of human genomic DNA as template. Real-time PCR was performed on the Corbett Rotor-Gene 6000 with 3 minutes of denaturation at 95°C, followed by 40 cycles of denaturation at 95°C for 1 second and combined annealing/extension at 60°C for 30 seconds. A melting curve was performed upon completion of cycling (65°C to 95°C, in 0.5°C increments).

Testing was performed on the following samples of KAPA SYBR FAST Universal 2x qPCR MasterMix:

1. Stored at 4°C for up to 15 days, tested at 12-hour intervals
2. Stored at 37°C for up to 15 days, tested at 12-hour intervals
3. Subjected to up to 40 freeze-thaw cycles, tested after every five cycles
4. Exposed to light (at 4°C) for up to 76 hours, tested at 2 or 3-hour intervals

In addition, a sample of KAPA SYBR FAST stored at the recommended -20°C for approximately 1 year was tested with the standard KAPA SYBR FAST quality control assay, which consists of qPCR of the human β-actin gene, with a ten-fold serial dilution of human genomic DNA (from 16 ng to 1.6 pg per 20 µL reaction) as template. Reaction components were the same as those used for the abovementioned assay. Results of this experiment were then compared to quality control data generated on the day of production of this batch of KAPA SYBR FAST Universal 2x qPCR MasterMix. Amplification plots are shown on subsequent pages. Melt curve data is not shown.

The results indicate that KAPA SYBR FAST 2x Universal MasterMix is stable at 4°C and 37°C for at least 15 days, and can withstand at least 40 freeze-thaw cycles. A gradual decrease in fluorescence can be seen when samples are exposed to light for more than 20 hours, which results in a slight decrease in Ct scores. When stored as recommended (-20°C, protected from light) the product is stable for at least 1 year.

We recommend that KAPA SYBR FAST qPCR Kits be stored at -20°C, with no more than 30 freeze-thaw cycles, and minimal exposure to light. Under these conditions, the mix is stable for at least 6 months.

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Figure 1: Amplification plot (logarithmic scale) of KAPA SYBR FAST Universal 2x qPCR MasterMix stored at 4°C for up to 15 days

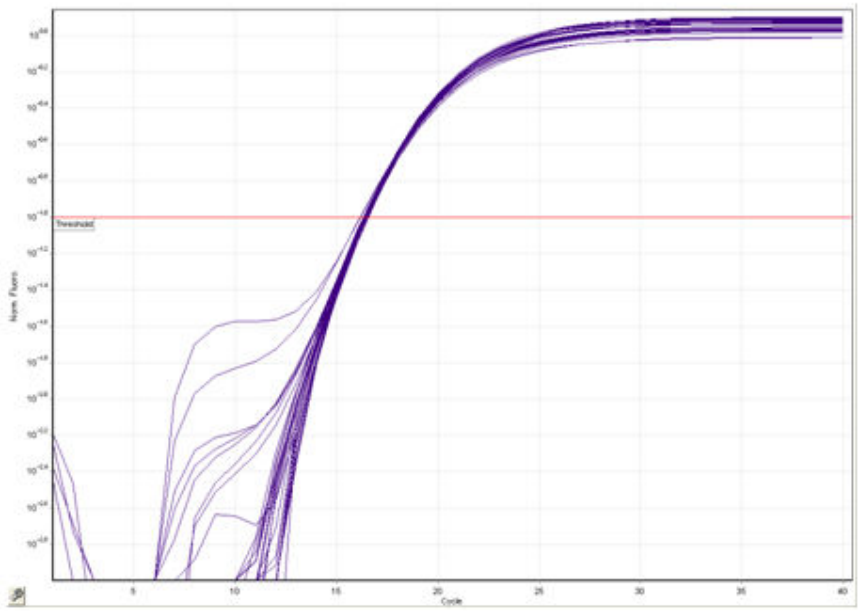


Figure 2: Amplification plot (logarithmic scale) of KAPA SYBR FAST Universal 2x qPCR MasterMix stored at 37°C for up to 15 days

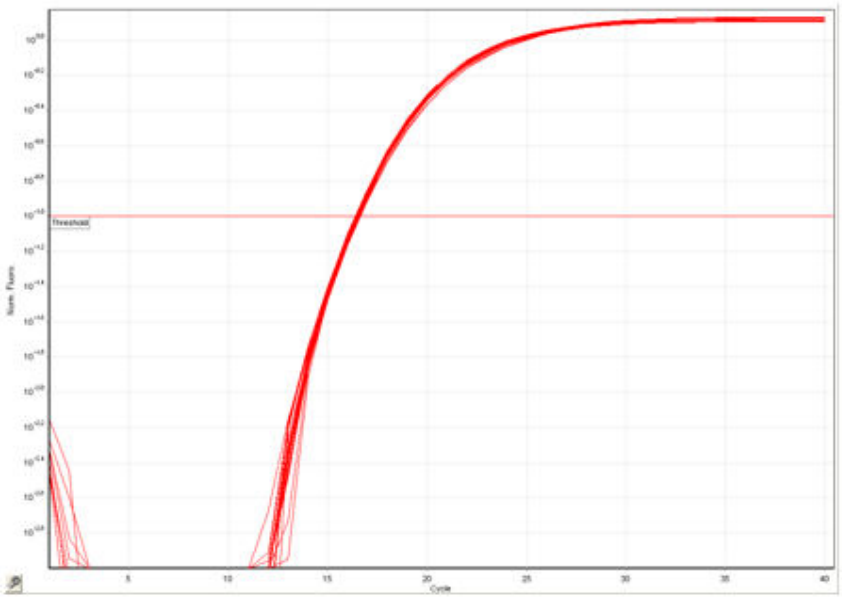
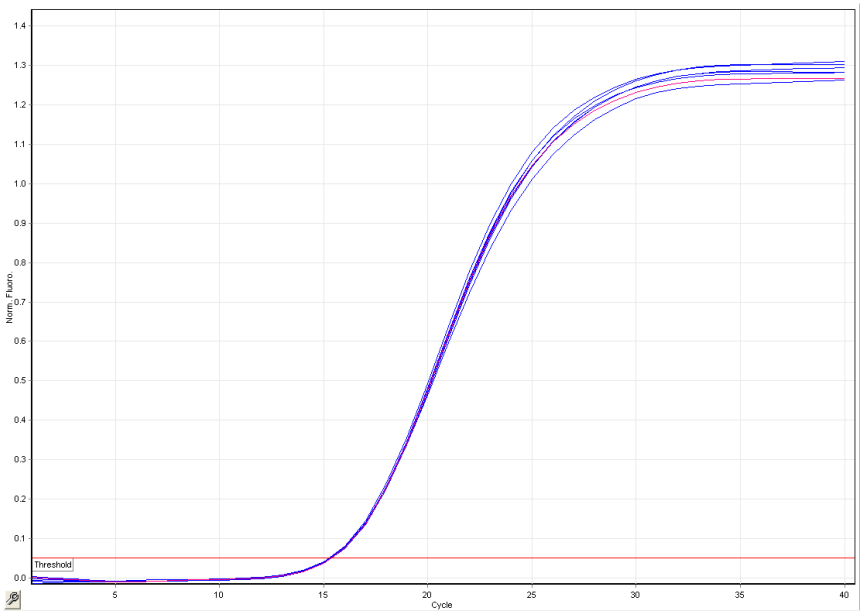
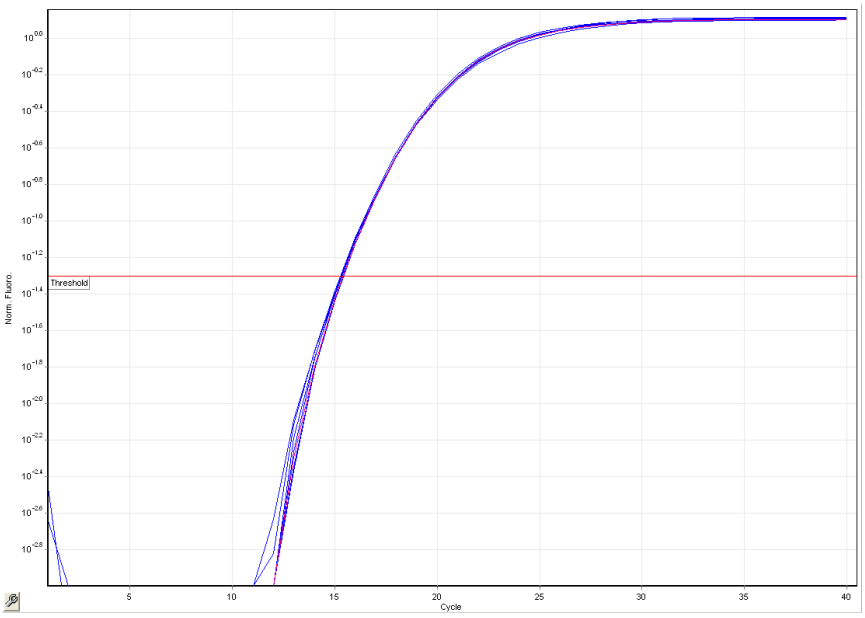


Figure 3: Amplification plots of KAPA SYBR FAST Universal 2x qPCR MasterMix subjected to up to 40 freeze-thaw cycles

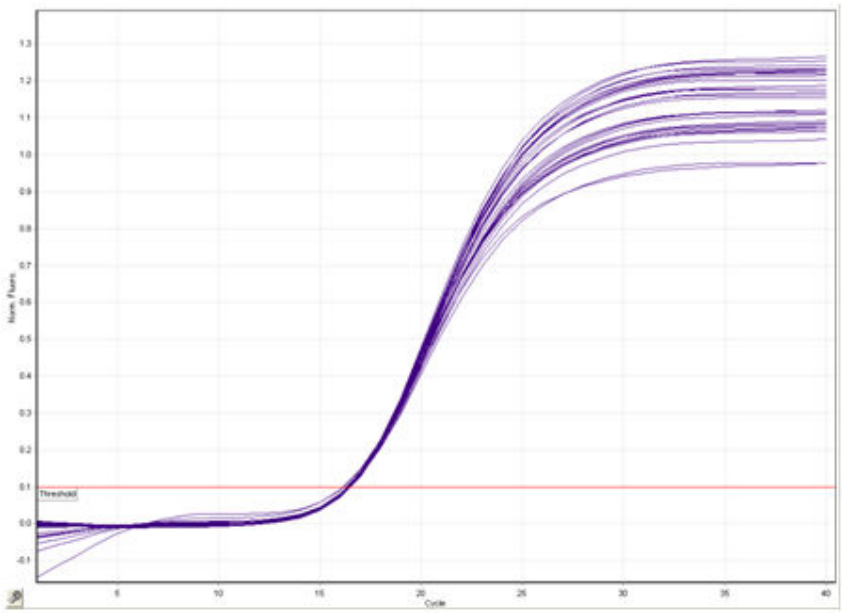


A: Linear Scale

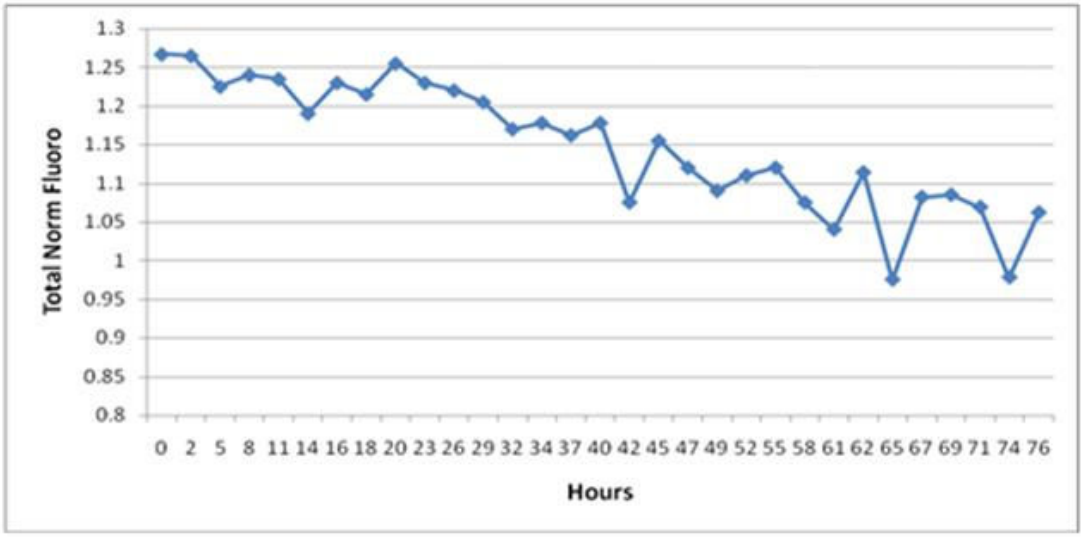


B: Log scale

Figure 4: KAPA SYBR FAST Universal 2x qPCR MasterMix exposed to light for up to 76 hours

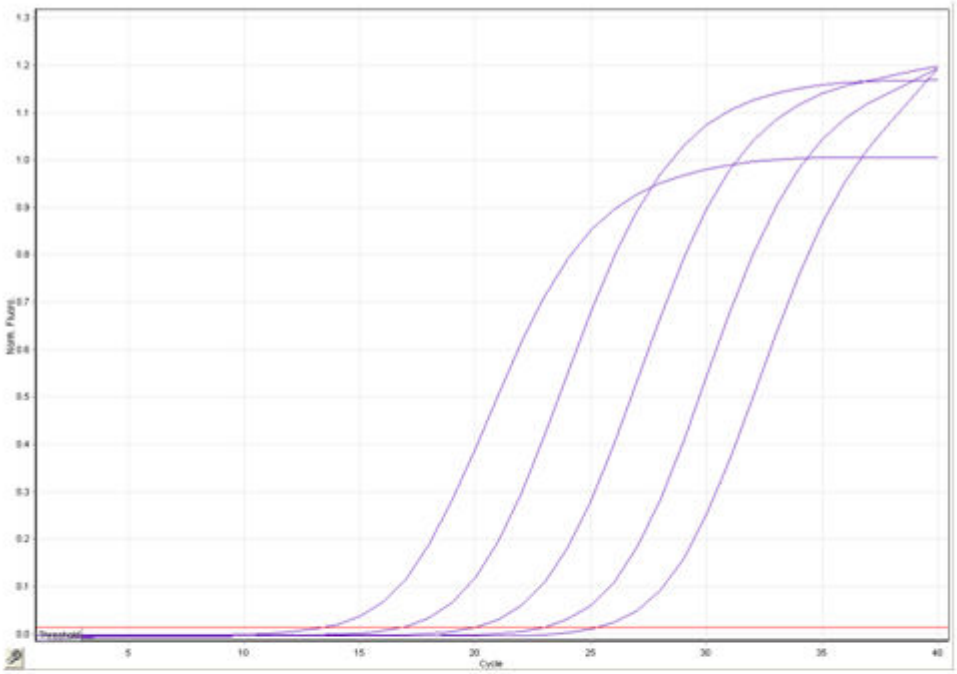


A: Amplification plot (linear scale)

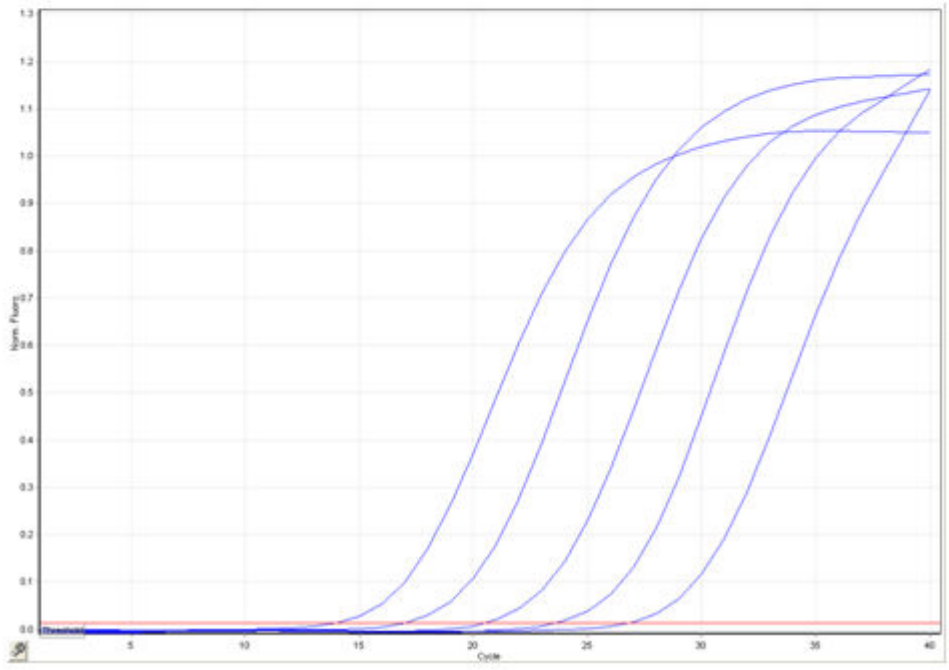


B: Effect of light exposure (in hours) on total normalized fluorescence

Figure 5: KAPA SYBR FAST tested on day of production and after 12 months at -20°C



A: Day of production



B: Stored at -20°C for approximately 12 months