Fast EvaGreen® qPCR Master Mix

Suitable for both qPCR and HRM with unrivaled performance

Amplification of Human Genomic DNA

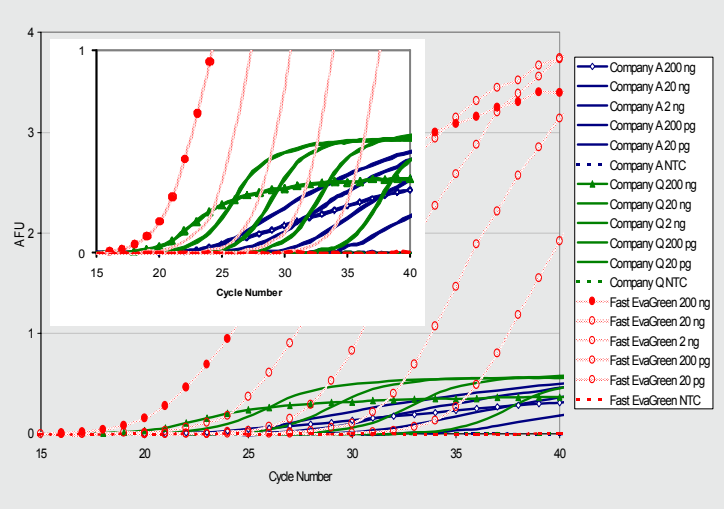

Figure 1. Comparison among Fast EvaGreen® master mix from Biotium and two fast SYBR® Green master mixes from two leading companies (company A and company Q) in the amplification of ATPG fragment (a 104-bp fragment encoding ATP gene) of human genomic DNA.. Reactions were carried out on the same plate using ABI 7900 Fast on DNA inputs of 200 ng, 20 ng, 2 ng, 200 pg, 20 pg and 0 pg, respectively. The passive reference dye, ROX, was adjusted for each master mix so that the final ΔRn faithfully reflects the fluorescence change of each master mix. To accomodate the relatively slow activation of company Q master mix, the entire plate was activated at 95 °C for 5 minutes. The cycling was carried out between 96 ° C (5 s) and 60 °C (30 s). Data set from the 200 ng DNA input are highlighted in the plot for easy distinguishing from the remaining amplification curves. The inset is an enlarged view of the area near the baseline for better viewing the curve patterns of the much weaker signals of the two SYBR-based master mixes.

Key Features

- √ **Superior sensitivity and wide dynamic range**
- √ **Suitable for both qPCR and HRM**
- √ **Compatible with both fast and regular cycling protocols**
- √ **Novel chemically-modified hotstart Taq requiring only 2 minutes to activate**
- √ **Suitable for TaqMan® assay as well, enabling multi-detections by both EvaGreen dye and oligo probes, followed by melt curve analysis**

The Technologies

Fast EvaGreen® qPCR master mix is a PCR reagent suitable for both qPCR and high resolution melt curve (HRM) analysis. The reagent system uses two of our breakthrough PCR technologies, EvaGreen® qPCR dye and Cheetah™ Taq hotstart DNA polymerase, to deliver superior results compared to other commercial master mixes.

- **EvaGreen® Dye:** EvaGreen® dye is the next generation DNA-binding dye ideal for both real-time PCR detection and HRM. The dye selectively binds to dsDNA via a novel "release-on-demand" mechanism that ensures low PCR inhibition and permits HRM application at below saturation dye concentration. Because the dye is spectrally similar to FAM or SYBR® Green I, it is compatible with all commercial qPCR instruments. Moreover, EvaGreen® dye is nonmutagenic and extremely stable.

- **Cheetah™ Taq:** Cheetah™ Taq is a new, chemically modified hotstart Taq DNA polymerase superior to AmpliTaq Gold®. Prepared from Taq and a proprietary Hot-off™ chemical modifier, Cheetah™ Taq can be activated in as little as two minutes under the standard hotstart condition (*i.e.*, 94 °C in pH 8-9 Tris) with high activity recovery. Additionally, Cheetah™ Taq is more stable at -20 °C or 4 °C than AmpliTaq Gold®, ensuring that the master mix maintain its peak performance following storage at low temperature. Cheetah™ Taq is more advantageous than any antibody-based hotstart polymerase, such as Platinum® Taq, because it is completely inactive at room temperature and also intrinsically less susceptible to DNA contamination.

The Performance

Fast EvaGreen® master mix has been tested on a variety of templates, including templates that are short (<100 bp), long (>700bp), AT-rich, GC-rich or of genomic origin. The results show, Fast EvaGreen® master mix is highly robust and superior to SYBR-based master mixes from other suppliers. Figures 1 and 2 compare Fast EvaGreen® master mix from Biotium with SYBR-based fast qPCR master mixes from two leading companies (company A and company Q) in the amplifications of human genomic DNA and human brain cDNA, respectively. The results demonstrate that Fast EvaGreen® Master Mix is significantly more sensitive and gives a wider linear detection range. The advantage of Fast EvaGreen® master mix is especially striking for the more challenging amplification of human genomic DNA (Figure 1); the EvaGreen® master mix gives early Ct values and a wide 5-log linear detection range while the master mix from company Q and, in particular, the master mix from company A suffer from significant Ct delay and narrow linear detection range (as suggested by the irregular spacings between the curves). Results on melt curve analysis mirror those on the qPCR for the three master mixes as shown in Figures 3 and 4; melt curves with Fast EvaGreen® master mix are far stronger than those with the two other SYBR-based fast master mixes.

Amplification of Human cDNA

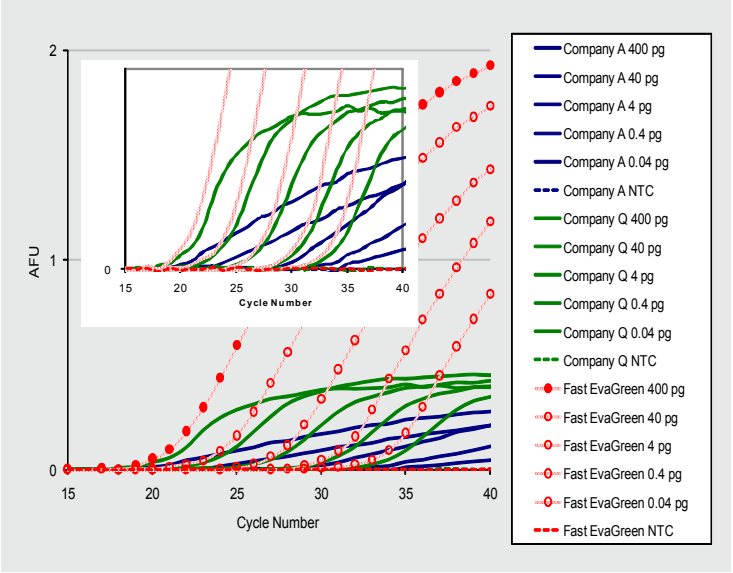

Figure 2. Comparison among Fast EvaGreen® master mix from Biotium and two fast SYBR® Green master mixes from two leading companies (company A and company Q) in the amplification of a B2M fragment (a 71-bp fragment encoding B2M transcript) of human brain cDNA.. Reactions were carried out on the same plate using ABI 7900 Fast on DNA inputs of 400 pg, 40 pg, 4 pg, 0.4 pg, 0.04 pg and 0 pg, respectively. The passive reference dye, ROX, was adjusted for each master mix so that the final ΔRn faithfully reflects the fluorescence change of each master mix. To accomodate the relatively slow activation of company Q master mix, the entire plate was activated at 95 °C for 5 minutes. The cycling protocol was: 96 °C for 5 s, 60 °C for 5 s and 72 °C for 25 s. The inset is an enlarged view of the area near the baseline for better viewing the curve patterns of the much weaker signals of the two SYBR-based master mixes.

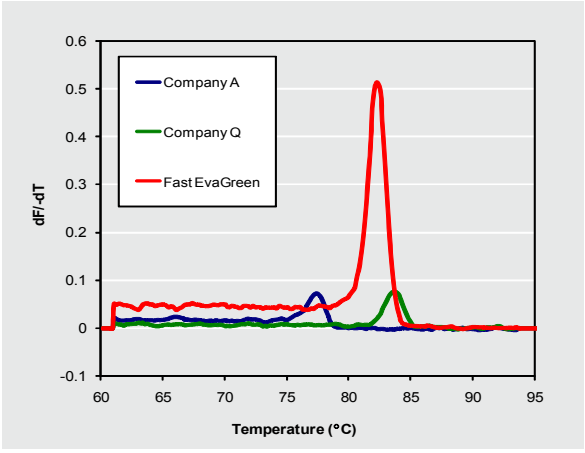

Figure 3. Comparison of Fast EvaGreen® master mix and fast qPCR master mixes from company A and company Q in melt curve analysis. Analyses were carried out following the amplification of 200 ng of human genomic DNA using each of the three master mixes, respectively, as detailed in Figure 1 legend.

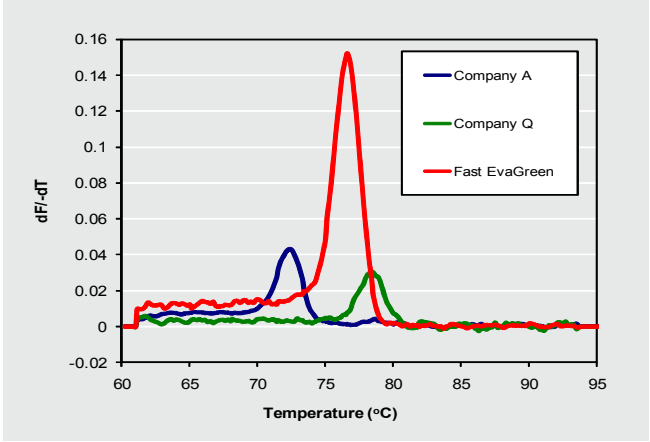

Figure 4. Comparison of Fast EvaGreen® master mix and fast qPCR master mixes from company A and company Q in melt curve analysis. Analyses were carried out following the amplification of 400 pg of human brain cDNA using each of the three master mixes, respectively, as detailed in Figure 2 legend.

The Versatility

In addition to its qPCR application, Fast EvaGreen® master mix can also be used for high resolution melt (HRM) analysis and oligo probe-based qPCR assay. HRM is a recently developed DNA analysis technique capable of detecting single mutations. It is gaining rapid popularity due to its simplicity and relatively low cost. An essential requirement for successful HRM is that the concentration of the DNA-binding dye be above or well above the optimal dye concentration used in qPCR for SYBR® Green I and other similar DNA binding dyes. As a result, for these dyes it is relatively difficult to formulate a single master mix optimal for both applications. Although some of the commercial master mixes using SYBR® or other dyes are promoted to be applicable for both qPCR and HRM, their performance usually suffers because a compromise has to be made in selecting a dye concentration sufficient for both applications. Since EvaGreen® dye has very low PCR inhibition because of its novel "release-on-demand" DNA-binding mechanism, a relatively high dye concentration can be used in qPCR for maximal signal strength. This same dye concentration is also ideal for HRM, resulting in a single master mix optimal for both applications.

Another unique application of Fast EvaGreen® master mix is qPCR detection by both EvaGreen® dye and one or more oligo probes (*e.g.*, TaqMan® probes or our AllGlo probes), all in the same reaction well. In this technique, EvaGreen® dye monitors total DNA production in the green channel while the oligo probes detect amplifications of individual targets in the red channels. Moreover, post PCR melt curve analysis can be performed to confirm the identities and number of products amplified. Figures 5-7 illustrate the utilities of Fast EvaGreen® master mix in these additional applications.

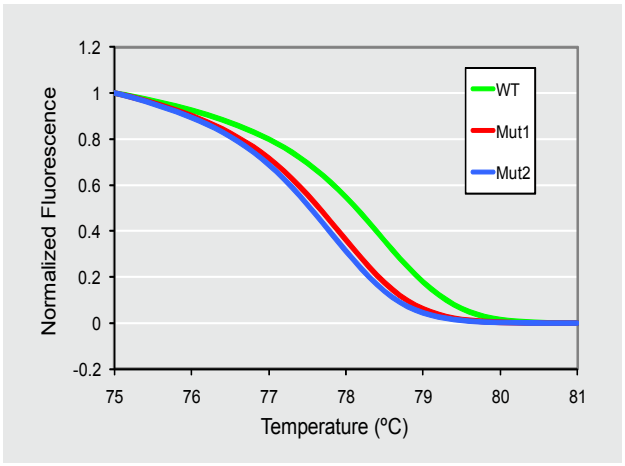

Figure 5. HRM analyses on products generated in Figure 4. Data are plotted in the form of normalized fluorescence vs. temperature (top plot) or fluorescence difference vs. temperature (bottom plot). In both plots, only one representative curve for each genotype is presented. HRM successfully resolved all three genotypes.

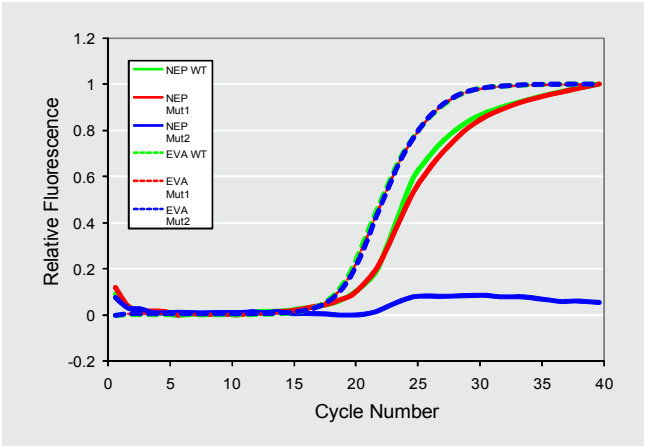

Figure 4. Amplifications of B2M gene fragment and its two mutants mutant 1 and mutant 2, respectively, using Fast EvaGreen® master mix containing both EvaGreen® dye and a NEP-labeled AllGlo™ probe (a TaqMan-like fluorogenic oligo probe). Mutant 1 has a single point mutation outside the probe hybridization region; Mutant 2 has a single point mutation in the middle of the probe. In each amplification, EvaGreen® signal (dashed lines) is recorded in the green (FAM) channel and the probe signal (solid lines) in the red (Cy5) channel. The wild type (green) and mutant 1(red) each produced positive signals in both FAM (dashed)and Cy5 (solid) channels. Mutant 2 (blue) produced a positve signal only in the FAM channel but essentially no positive signal in the Cy5 channel due to failure in probe hybridization.

The Value

Fast EvaGreen® master mix is among the most competatively priced qPCR master mixes on the market. Coupled with its unmatched performance, Fast EvaGreen® master mix offers you the best value. We can afford to offer you the competitive price because we are the original inventors of the two key technologies in the product: EvaGreen® qPCR dye and Cheetah™ hotstart Taq DNA polymerase.

Fast EvaGreen® master mix is available in these packaging sizes:

- **Cat# 31003, 200 reactions**
- **Cat# 31003-1, 500 reactions**
- **Cat# 31003-2, 5,000 reactions**

Reaction volume is 20 µL/well. ROX reference dye is provided in separate vials. A detailed protocol is included with each product and can also be downloaded at Biotium website.

You may also be interested in the following related products:

Cat. #	Product Name	Unit Size	Application
31000	EvaGreen dye, 20X in H_2O	5x1 mL	EvaGreen dye 20X concentrated in ultra pure H_2O is specifically formulated for qPCR application. Please ask for licensing opportunity.
29050	Cheetah™ hotstat Taq DNA polymerase	500 rxn	Cheetah Taq is a hotstart DNA polymerase prepared by covalently attaching a proprietary chemical modifier to Taq. Taking only 2 minutes to activate and with its excellent stability during storage, this chemically modified hotstart Taq is superior to AmpliTaq Gold. It is also more advantageous than antibody-based hotstart enzymes due to intrinsically low DNA contamination and complete lack of enzyme activity at room temperature. Various packaging sizes are available. Please ask for licensing opportunity.
29051	EvaEZ Fluorometric DNA Polymerase Activity Assay (200rxn)	2x1 mL	This kit provides a safe alternative to the traditional hazardous radioactive method for assaying DNA polymerase activity. The fluorescence-based method is highly senstive and simple, requiring only a fluorometer, microplate reader or realt-time PCR instrument to perform.
40013	PMA™ DNA modification agent	1 mg	PMA is a cell membrane-impermeable DNA modification dye that can be used to selectively covalently modify DNA from dead cells, rendering the modified DNA unamplifiable. Subsequently, DNA from viable cells can be quantified by qPCR. Thus, PMA can be used in selective detection of viable pathogens in the presence of dead pathogens by qPCR.
41003	GelRed™ nucleic acid gel stain, 10,000X in H_2O	0.5 mL	GelRed is the perfect replacement for the highly toxic ethedium bromide (EB) for nucleic acid gel staining. It is far more senstive than EB but is nonmutagenic, noncytotoxic and safe to dispose in the drain. The dye is spectrally similar to EB, so it is completely compatible with existing instruments.
41005	GelGreen™ nucleic acid gel stain, 10,000X in H_2O	0.5 mL	Similar to GelRed, GelGreen is a nucleic acid gel stain with superior senstivity and excellent safety profile. GelGreen is ideal for visible light excitation, which avoids DNA damage by UV light.

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