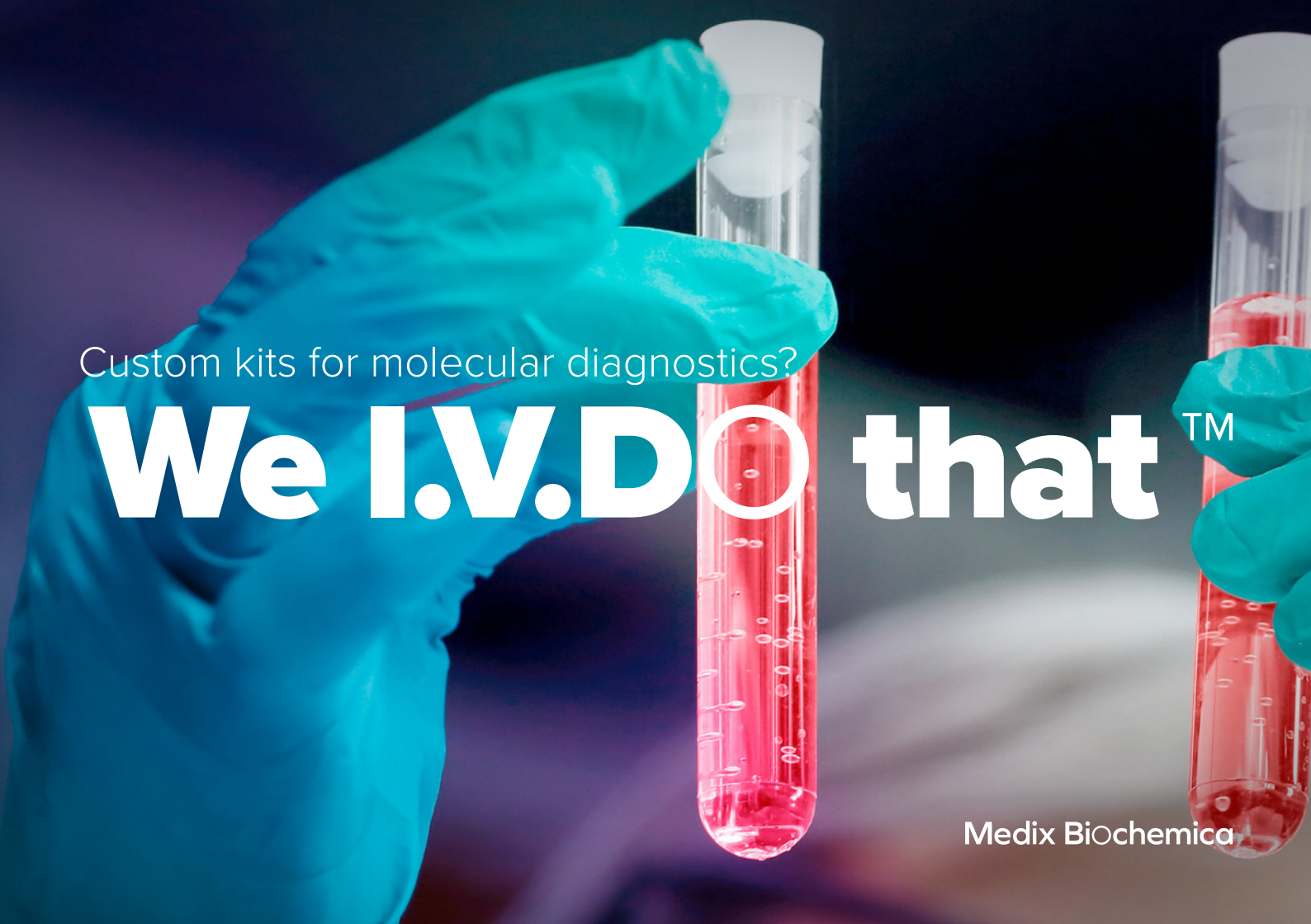
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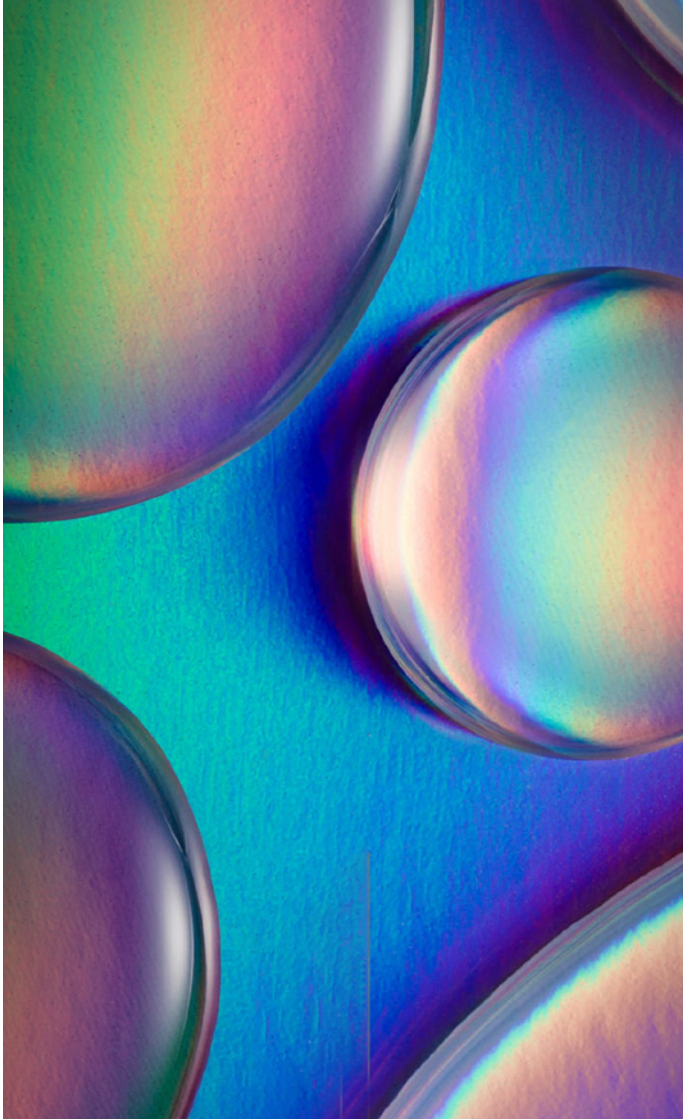
MX2106 MedixMDx Fast Bst Mix

A simple, ready-to-use mix containing a recombinant DNA polymerase expressed by *Geobacillus stearothermophilus*.

The *Bst* DNA polymerase displays high strand displacement activity, exhibits 5’ to 3’ polymerase activity, but lacks 5’ to 3’ exonuclease activity. MedixMDx Fast *Bst* Mix is suitable for isothermal nucleic acid amplification methods such as loop-mediated isothermal amplification (LAMP).

Isothermal LAMP amplification is commonly used for end-point analysis. Real-time detection is also possible if a suitable dye-system is added to the mix.



MedixMDx Fast *Bst* Mix is a ready-to-use mix that includes an advanced buffer system to ensure high yield and performance – only primers, template, and water need to be added. It is highly tolerant to inhibitors, enabling rapid and robust LAMP reactions with difficult crude sample matrices, e.g. blood. The typical reaction temperature is 65°C; however, the enzyme is active at a wide range of temperatures from 55°C to 70°C. The enzyme can be inactivated at temperatures higher than or equal to 80°C. MedixMDx Fast *Bst* Mix is sold with a separate fluorescent intercalating dye, which allows real-time detection of LAMP reactions using a qPCR instrument on FAM channel.

Methods

MedixMDx Fast Bst Mix with fluorescent dye was compared with a leading supplier's warm start LAMP Mix. LAMP primers were designed against Lambda DNA (GeneBank NC_001416) as per Nagamine *et al*, 2002.[1] The study was performed on a DNA serial dilution of 53.2, 5.3, 0.5, 0.27 fg/rxn plus a no-template control (NTC). The 25 µL samples were incubated at 65°C for 50 minutes and inactivated at 95°C for 10 minutes in order to perform end-point analysis.

Real-time detection was performed using a BioRad CFX Opus 96 cycler with fluorescence readings taken every 15 s on the FAM channel. The reactions were then visualized using gel electrophoresis with 2% Invitrogen E-gel agarose gels.

The study was performed using two reaction conditions: optimal reaction conditions as supplied in a kit, or in the presence of a strong PCR inhibitor, blood, at 0.5% or 2% v/v in the final reaction mix.

The longer experiment time of 50 minutes was selected in this study for investigative purposes only; typically, reactions are run for 30 minutes.

Fast and sensitive

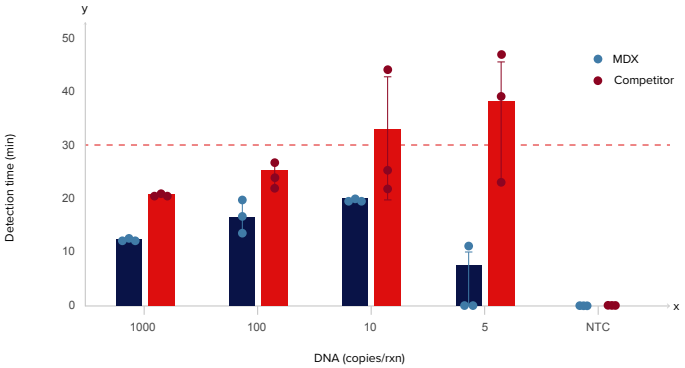
The target detection times are approximately 10–20 minutes depending on the initial DNA quantity (Figure 1). This is on average 10 minutes faster than the detection time with the competitor's mix.

The assay sensitivity with MedixMDx Fast *Bst* Mix is approximately 10 target DNA copies/reaction (0.5 fg/rxn), showing higher sensitivity than the competitor's mix, where not all replicates are amplified at this DNA concentration within 30 minutes.

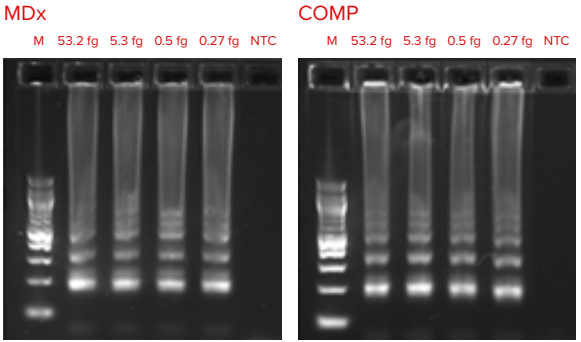
Target DNA concentrations below 10 copies/rxn may yield inconsistent or non-reproducible results, with amplification occurring after 30 minutes.

Figure 1. MedixMDx Fast *Bst* Mix vs. competitor's warm start mix, run in optimal reaction conditions.

A. Normalized detection time calculated from recorded fluorescence signals.



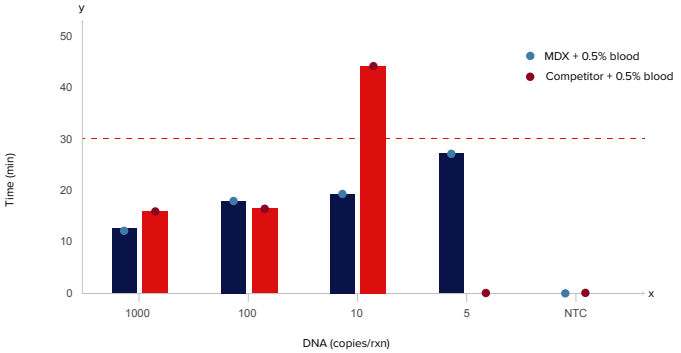
B. Results obtained from gel electrophoresis.



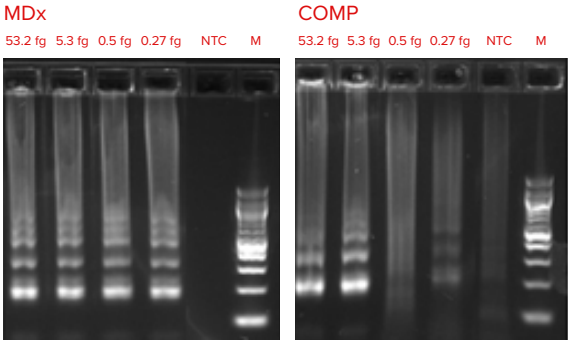
The presence of diluted blood at 0.5% v/v per reaction does not impact the MedixMDx Fast *Bst* Mix sensitivity or detection time, which remains at 10–20 minutes for the tested DNA concentrations. The competitor's mix performed weaker in the presence of 0.5% blood, with the sensitivity dropping to 100 copies/rxn (Figure 2).

Figure 2. MedixMDx Fast *Bst* Mix vs. competitor's warm start mix, run with 0.5% blood/reaction.

A. Normalized detection time calculated from recorded fluorescence signals.



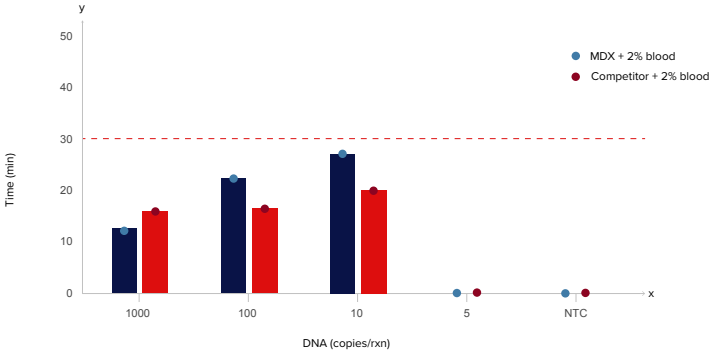
B. Results obtained from gel electrophoresis.



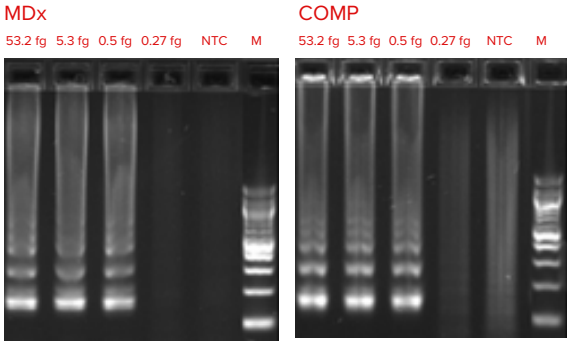
Amplification within a complicated sample matrix, e.g. 2% v/v blood (0.5 µl of undiluted blood/ reaction), increases the target detection time for MedixMDx Fast *Bst* Mix minimally, by 2–4 minutes, in comparison with normal samples. The assay sensitivity is not impacted (Figure 3).

Figure 3. MedixMDx Fast *Bst* Mix vs. competitor's warm start mix, run with 2% blood/reaction.

A. Normalized detection time calculated from recorded fluorescence signals.



B. Results obtained from gel electrophoresis.



Conclusions

MedixMDx Fast *Bst* Mix is a fast and sensitive isothermal DNA polymerase, which displayed superior performance over another *Bst* mix from a leading supplier.

MedixMDx Fast *Bst* Mix is not deterred by complex reaction matrices, it is able to amplify DNA targets in the presence of 2% v/v blood without slowing down the detection time.

Contact us

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1. Nagamine *et al.* Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and Cellular Probes*. 2002;16:223–229.