

Application note

KASP Master Mix Triton-free formulations: complying with new regulations

Introduction

The [European Chemicals Agency](#) (ECHA), who oversee the safe use of chemicals, established the [REACH regulations](#) in 2007 for the Registration, Evaluation, Authorisation and Restrictions of Chemicals. REACH aims to improve the protection of human health and the environment from risks that can be posed by chemicals.

In November 2017, several classes of chemicals were added to the REACH regulations which prohibit their use, production and sale within the European Union (EU). One chemical included was the detergent IGEPAL CA-630, which is also known by the trade name Triton™ X-100. By January 2021, manufacturing processes requiring Triton X-100 (hereafter referred to as Triton) will be prohibited within the UK/EU.

The entire catalogue of [KASP™ Master Mixes](#) manufactured by LGC, Biosearch Technologies™ was originally formulated with Triton. KASP Master Mix is central to our patented PCR-based [KASP genotyping technology](#), which serves as a critical tool for plant and livestock breeders, as well as in other research areas including human genotyping studies. Biosearch Technologies made the decision to reformulate all KASP Master Mixes to ensure their manufacture could continue within the UK/EU in 2021.

During the update process, the Triton in the KASP Master Mixes was replaced with an alternative detergent, resulting in [Triton-free \(TF\) Master Mixes](#).

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To confirm that this formulation change did not impact the function of the KASP Master Mixes, our scientists performed an extensive validation project which ran over 850 PCR plates and generated over half a million data points. An array of tissue types, extraction methods, genotyping platforms and KASP assays were included in the validation to represent the wide-ranging interests of the scientists working with

KASP genotyping chemistry.

This application note outlines the robust testing that was completed to confirm that KASP-TF Master Mix formulations have equivalent performance to legacy formulations and no impact on the end-point genotyping data produced.

KASP-TF Master Mix validation in numbers

Number of tissue types	6
Number of extraction methods	4
Number of sample sets (tissue type + extraction method)	10
Number of genotyping platforms	3
Number of unique samples	986
Number of plates and arrays run	862
Total number of data points	585,600

Materials and methods

To validate the KASP-TF formulations, the same genotyping reactions, using the same template DNA and the same KASP assay mixes, were performed in parallel using KASP-TF Master Mix and legacy KASP Master Mix formulations. End-point genotyping data was plotted on Cartesian (cluster) plots, and inspected to directly compare the performance of both formulations. Ten different sample sets were compared, incorporating the range of tissue types and extraction methods used.

Tissue types

Seeds from three popular crop species were selected, namely *Zea mays* (maize), wheat (*Triticum aestivum*) and tomato (*Solanum lycopersicum*). *Helianthus annuus* (sunflower) seeds were also included in the validation due to their high oil content, hence would provide material to test that new TF formulations were equally as inhibitor-tolerant as legacy formulations. Leaf material from *Lactuca sativa* (lettuce) was selected as the representative leaf tissue. In total, DNA was extracted from over 1,000 plant samples within the validation project.

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Blood is another popular starting material for (non-plant) genotyping studies and, importantly, many of the best characterised KASP assays target human single nucleotide polymorphisms (SNPs). By incorporating human DNA samples, the validation could include a selection of KASP assays that would facilitate the identification of subtle performance differences.

DNA extraction

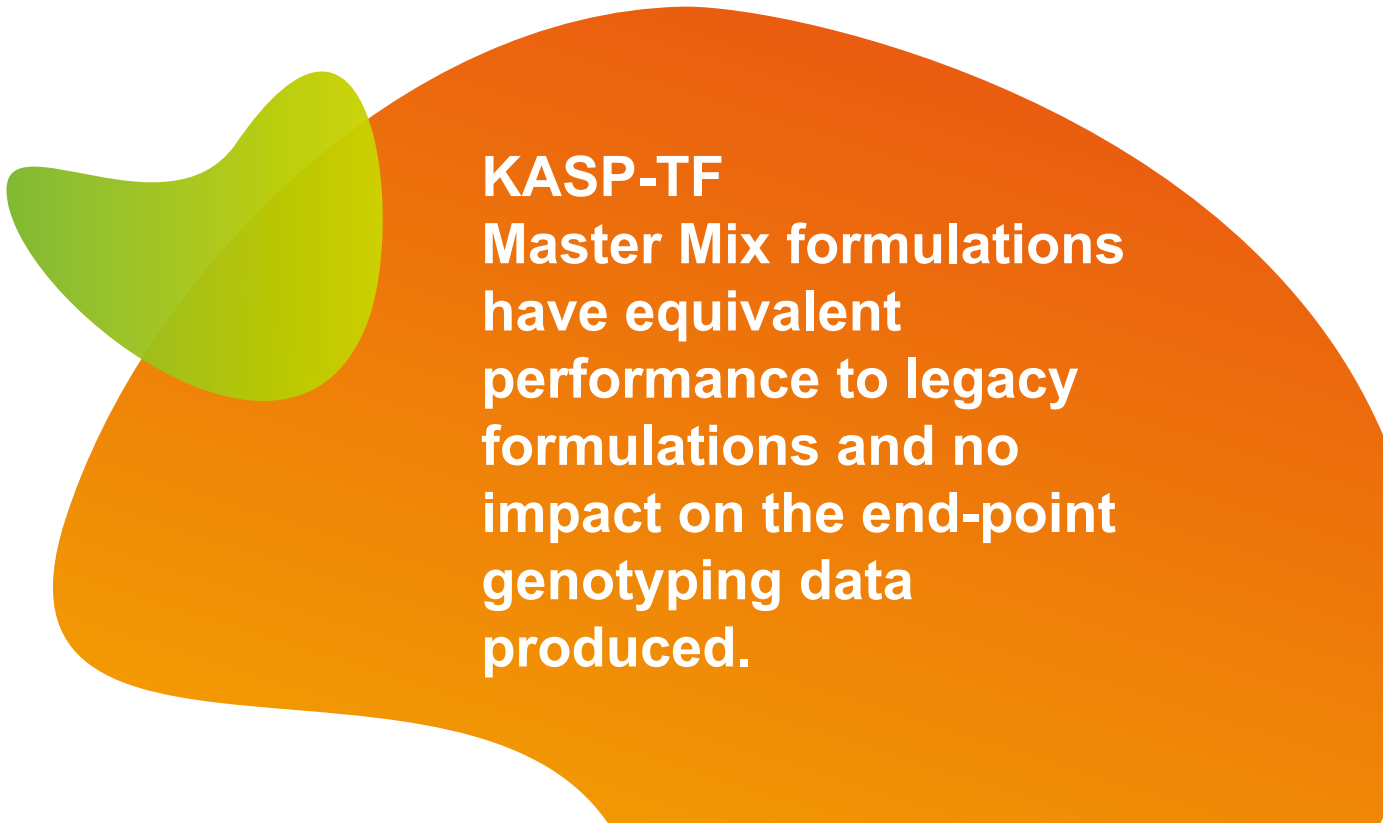
A wide range of DNA extraction methodologies were included in the validation study. Biosearch Technologies' purification technologies [sbeadex™](#) and [Kleargene™](#) were used for plant and human blood samples, whilst [QuickExtract™](#) extraction chemistry was used for plant samples. These methods were performed following the protocols detailed in the relevant product user guides. A standard crude NaOH 'HotShot' DNA extraction method was also tested to establish that the quality of DNA would not affect the performance of TF formulations compared to legacy formulations.

Genotyping platforms

Extracted DNA was genotyped using our KASP genotyping technology in 384-well plates, 1536-well plates and in [Array Tape™](#). The reaction plates were processed using our in-house [SNPline™](#) workflow, and thermal cycling was performed in the [Hydrocycler²™](#). Reactions set up in Array Tape were processed using the [Nexar™](#), our modular inline liquid handling and assay processing system.

KASP Master Mix and KASP assays

The validation tested three types of the new KASP-TF Master Mix. These were: a) KASP-TF v4 96/384-well Master Mix (formulated for reactions performed in 96-well and 384-well plates); b) KASP-TF v4 1536-well Master Mix (optimised specifically for smaller reaction volumes), and; c) KASP-TF v5 Master Mix (optimised for high-throughput applications and used with Array Tape on the Nexar platform).



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Each TF formulation was validated by comparing its performance to the equivalent legacy formulation using a wide selection of [KASP genotyping assays](#). KASP assay mix is specific to the SNP or Insertion/Deletion (InDel) to be targeted and consists of two competitive allele-specific forward primers and one common reverse primer.

For each species included in the validation (see tissue types section above), a minimum of eight KASP assays were selected. The KASP assays that were selected used primers with a wide array of different percentage GC contents, ranging from 21-72%. A number of the assays required optimised cycling conditions rather than the standard 61-55 °C touchdown thermal cycle.

Validation criteria

Strict criteria were used to validate the new TF formulations against the legacy formulations of KASP Master Mix.

1. Qualitative inspection

All data plots that were generated (for 580,000 plus data points) were independently assessed by two senior scientists. This qualitative data inspection included assessment of cluster quality and position to determine if they were equivalent between the comparable TF and legacy mixes.

2. No template controls

No template controls (NTCs) are reactions that contain no DNA and therefore should not amplify during the PCR. On a genotyping plot, these data points are expected to plot at the origin, indicating no fluorescent signal has been generated. In each comparison experiment, the number of TF formulation NTCs that migrated from the origin had to be less than or equal to the number of legacy formulation NTCs that migrated, or within a defined percentage difference. For example, if within a data set that included eight NTCs, there were more than two NTCs that showed a difference then the TF formulation would not pass.

3. Call rate

The call rate refers to the percentage of data points that can be assigned a genotype. To pass validation, the percentage call rate for the TF formulation had to be equal to or greater than the percentage call rate for the comparable legacy formulation in each experiment. If the call rate was lower for the TF formulation, a defined percentage point difference was permitted (e.g. 5% for 1536-well plates).

4. Data concordance

This refers to the concordance of the genotype calls between the TF and legacy formulations within an experiment. Data concordance for TF formulations had to be at least 97% with legacy formulations.



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Results and discussion

Genotyping results

Figure 1 illustrates representative cluster plots (A, B and C) for three experiments within the validation. These plots represent the broad range of tissue types (leaf, seed and blood), DNA isolation chemistries (QuickExtract, sbeadex and Kleargene), and reaction formats (384-well, 1536-well and Array Tape) that were considered in the validation. The data obtained

for the legacy KASP Master Mixes (Batch A and Batch B) and for the KASP-TF Master Mix is highly comparable in each plot, which clearly illustrates that the change in formulation has had no impact on the data obtained. The extent of similarity shown in Figure 1 is representative of that observed throughout the entire validation project.

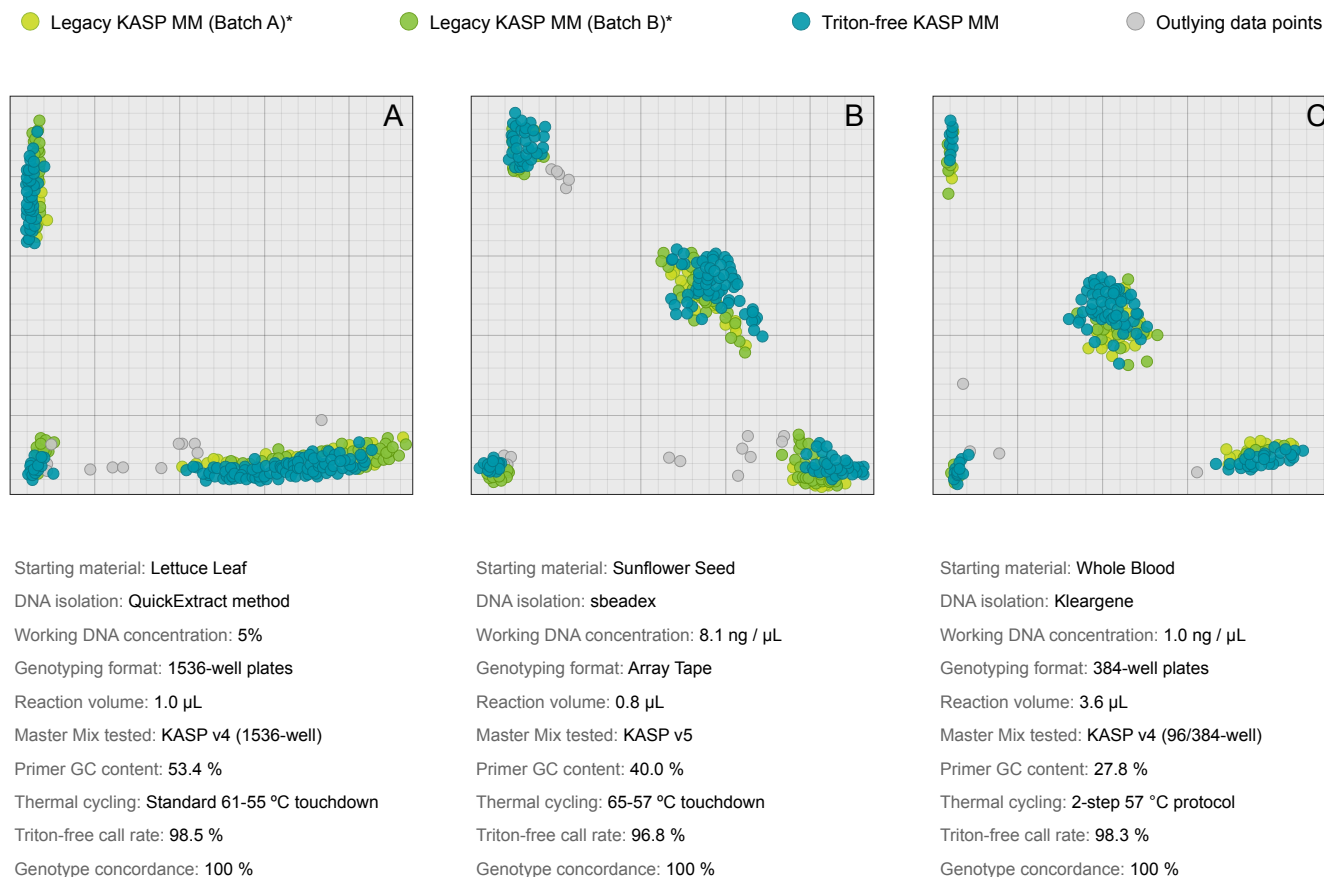


Figure 1. Representative cluster plots illustrating the similarity between data generated with Triton-free and legacy KASP Master Mix. In all three plots (A, B, and C), data for KASP-TF Master Mix shows equivalent performance to the data for legacy KASP Master Mix. These three plots were chosen to represent the range of starting materials, DNA isolation chemistries, reaction formats, and KASP assays included in the validation project.

*The plots show data from two batches of legacy KASP Master Mix; these batches have the same formulation but were manufactured at different times. The tight clustering of these two batches demonstrates the robustness of our mix manufacturing and QC processes.

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Table 1 summarises the data obtained for 384-well and 1536-well plates in the extensive validation experiments that were performed. Results are presented in terms of the number of plates that passed each validation criteria, for each of the tissue types, isolation methods

and KASP Master Mixes analysed. In all experiments, for all validation criteria, the new TF formulation of each KASP Master Mix demonstrated equivalent performance to the legacy formulation equivalent.

Starting material	DNA purification/extraction method	Master Mix	Cluster-plot assessment	Call rate assessment	Genotype concordance assessment	NTC signal assessment
Wheat grain	sbeadex	KASP-TF v4 ¹	✓ 8 plates passed (6 required)	✓ 8 plates passed (6 required)	✓ 8 plates passed (6 required)	✓ 8 plates passed (6 required)
		KASP-TF v5	✓ 15 plates passed ² (14 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)	✓ 14 plates passed (12 required)
Sunflower seed	sbeadex	KASP-TF v4	✓ 16 plates passed (14 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)
		KASP-TF v5	✓ 16 plates passed ² (14 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)
Tomato seed	sbeadex	KASP-TF v4	✓ 15 plates passed (14 required)	✓ 14 plates passed (12 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)
Tomato seed	QuickExtract	KASP-TF v4	✓ 16 plates passed (14 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)
Maize seed	Hot Shot	KASP-TF v4 ²	✓ 8 plates passed (7 required)	✓ 8 plates passed (6 required)	✓ 8 plates passed (6 required)	✓ 8 plates passed (6 required)
Lettuce leaf	sbeadex	KASP-TF v4	✓ 16 plates passed (14 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)
		KASP-TF v5 ¹	✓ 8 plates passed ² (7 required)	✓ 8 plates passed (6 required)	✓ 8 plates passed (6 required)	✓ 8 plates passed (6 required)
Lettuce leaf	QuickExtract	KASP-TF v4	✓ 16 plates passed (14 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)	✓ 16 plates passed (12 required)
		KASP-TF v5	✓ 15 plates passed ² (14 required)	✓ 15 plates passed (12 required)	✓ 15 plates passed (12 required)	✓ 15 plates passed (12 required)
Whole blood (Human)	Kleargene	KASP-TF v4	✓ 32 plates passed (30 required)	✓ 32 plates passed (30 required)	✓ 32 plates passed (30 required)	✓ 32 plates passed (30 required)
Whole blood (Human)	Kleargene	KASP-TF v4 with optimised cycling	✓ 31 plates passed (24 required)	✓ 31 plates passed (24 required)	✓ 31 plates passed (24 required)	✓ 31 plates passed (24 required)
		KASP-TF v5 with optimised cycling	✓ 31 plates passed ² (24 required)	✓ 31 plates passed (24 required)	✓ 31 plates passed (24 required)	✓ 31 plates passed (24 required)
Total			243 plates passed	243 plates passed	245 plates passed	243 plates passed

Table 1. Summary table of the validation data for the updated TF formulations of KASP Master Mix, in 384-well and 1536-well format. Equivalent performance was observed between TF and legacy formulations, with all experiments demonstrating sufficient numbers of passed plates for all validation criteria. The results shown are from 8x 384-well plates and 8x 1536-well plates except for the human genotyping experiments (which were run in duplicate), and other sample sets where indicated.

¹ Only the data from the 1536-well plate validation is included in the table.

² Only the data from the 384-well plate validation is included in the table.

Streamlining the range of KASP products

Biosearch Technologies utilised the opportunity of this essential reformulation to streamline the range of KASP Master Mix products in the portfolio. Several KASP legacy products have been discontinued or merged, which we

hope will simplify the product offering for our customers and make it easier to determine the appropriate mix for your application. Table 2 summarises the legacy KASP Master Mix formulations and the new TF equivalents.

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Legacy KASP Master Mix formulations	Triton-free KASP Master Mix formulations
KASP v3 (all)	KASP-TF v4 96/384
KASP v4 96/384	
US-specific KASP v4 96/384	
KASP v4 1536	KASP-TF v4 1536
US-specific KASP v4 1536	
KASP v5 Array Tape	KASP-TF v5
KASP for Fluidigm	KASP-TF for Fluidigm

Table 2. A summary of the legacy KASP Master Mix formulations and the new Triton-free equivalents in the streamlined KASP Master Mix portfolio.

Summary

Due to changes in the REACH regulations published by the European Chemicals Agency in 2017, Biosearch Technologies has made an essential change to the formulation of all KASP Master Mixes in the portfolio. The detergent Triton has been replaced and we have subsequently performed an extensive validation project to ensure that the updated formulations will continue to enable the critical work of scientists using KASP genotyping chemistry across the globe. The validation experiment included a broad representative range of species and tissue types, involved over 850 reaction plates and generated over 580,000 data points. The data analysed and presented here clearly demonstrate that the KASP-TF

Master Mixes perform equivalently to the legacy formulations and the formulation change has had no impact on the data generated. KASP-TF Master Mixes can therefore be introduced into any ongoing and future KASP genotyping experiments without any disruption to the workflow. In conclusion, Biosearch Technologies' new Triton-free formulation provides a complete range of KASP-TF Master Mixes for genotyping into 2021 and beyond.

Integrated tools. Accelerated science.

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