Triton-free KASP Master Mix formulations

Background and validation

Agenda

This presentation will cover:

- The reasons for the change
- What exactly is changing?
- The extensive testing performed
  - Tissue types
  - Extraction methods
  - Platforms
  - Assays
- Data to illustrate equivalent performance

The reasons for the change

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- European Chemicals Agency (ECHA) established REACH in 2007 for the **R**egistration, **E**valuation, **A**uthorisation and **R**estrictions of **C**hemicals.
- In November 2017, several classes of chemicals were added to REACH regulations, which prohibit use, production and sale within the EU.
- One chemical is the detergent IGEPAL CA-630, also known by the trade name Triton™ X-100.
- By January 2021, sales of products containing Triton X-100 will be prohibited within the EU.
- It is therefore necessary to reformulate several products to be Triton-free.

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What is changing?

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- All KASP™ Master Mix products have been reformulated
  - Triton replaced with a different detergent
  - Now Triton-free (TF)
- Several KASP legacy products have been discontinued or merged
  - Streamline the range of KASP products
- RepliKator™ wash, Kleargene™ and mag™ beads have also been reformulated
  - Now Triton-free (TF)

Legacy KASP MM formulations	Triton-free KASP MM 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

This table summarises the legacy KASP Master Mixes and the new Triton-free (TF) equivalents.

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Extensive testing of new KASP formulations

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- An **extensive validation** was performed on the Triton-free formulations
- Our scientists ran over **850 PCR plates**
- Generated over **580,000 data points**
- **Robust testing** to confirm that triton-free formulations have **equivalent performance** to legacy formulations across a range of:
  - Tissue types
  - Extraction methods
  - Platforms
  - Assays

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Triton-free KASP mix validation

Tissue types

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- Wide range of tissue types included in the validation:
  - **Seeds**
    - Maize
    - Wheat
    - Tomato

    } Popular crop species
    - Sunflower – oily seed, ensure new formulation is equally inhibitor tolerant
  - **Human blood** – many well characterised assays available
  - **Lettuce leaf** – representative leaf tissue
- Extracted DNA from over **1000 plant samples**

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Triton-free KASP mix validation

Extraction chemistries

- Wide range of extraction methods included in the validation
- Biosearch Technologies' extraction and purification chemistries:
  - **sbeadex**™ DNA purification
  - **Kleargene**™ DNA purification
  - **QuickExtract**™ Plant DNA Extraction Solution
- Crude extraction method:
  - NaOH "Hot Shot" DNA extraction

Triton-free KASP Master Mix validation

Platforms and assays

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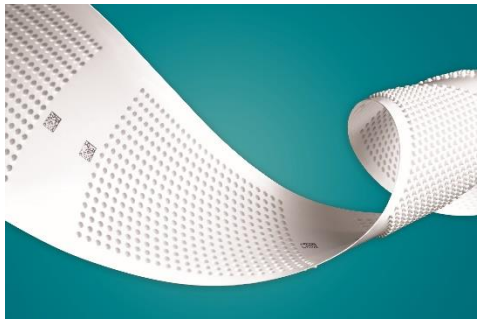
- Validation was performed on multiple platforms
  - SNPline™ – 384-well and 1536-well plates
  - Nexar – Array Tape
- A wide range of different assays were included in the validation
  - A minimum of 8 assays per tissue and extraction type
  - KASP primers with %GC content ranging from 21%-72%
  - Assays requiring optimised cycling conditions

	SNPLine 384-well plates	SNPLine 1536-well plates	Nexar Array Tape
KASP-TF v4 384	✓	N/A	N/A
KASP-TF v4 1536	N/A	✓	✓
KASP-TF v5	✓	✓	✓



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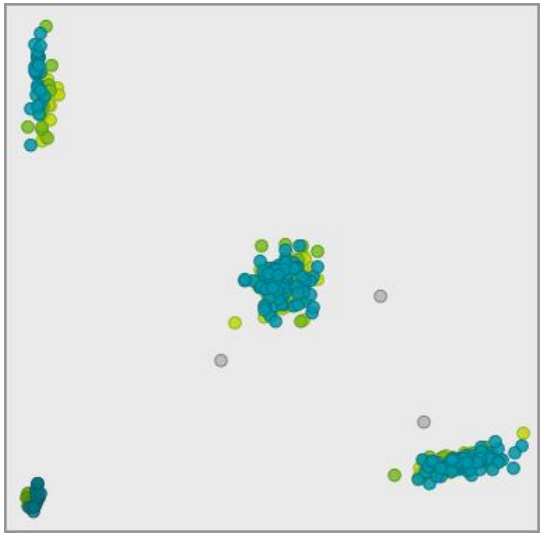
Triton-free KASP Master Mix validation

Strict criteria for mix to pass

- Qualitative inspection of plotted data
  - Independently assessed by 2 senior scientists
  - Determine if cluster quality and position were equivalent
- No-template controls (NTCs)
  - The number of TF-mix NTCs that migrate from the origin of the plot must be **less than or equal to** the number of legacy mix NTCs that migrate from the origin of the plot, or within a defined % difference
- Call rate
  - TF-mix ≥ legacy mix, or within defined %point difference
- Data concordance
  - TF-mix must have ≥97% concordance with legacy formulations



Validation Results – Example A

KASP genotyping using DNA extracted with QuickExtract Plant DNA Extraction Solution

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Starting material: **Lettuce Leaf**

DNA purification: **QuickExtract method**

Working DNA concentration:[1] **5%**

Genotyping format: **1536-well plates**

Reaction volume: **1.0 µL**

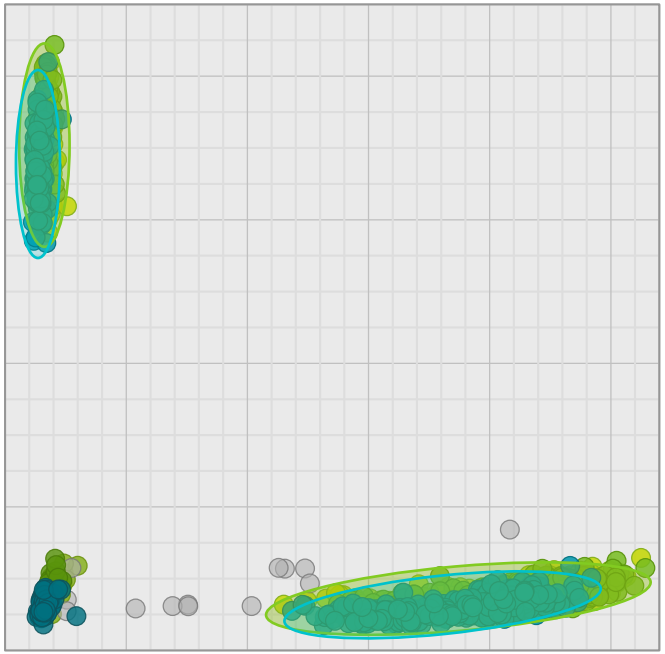
Master mix tested: **KASP v4 (1536-well)**

Primer GC content:[2] **53.4%**

Thermal cycling: **Standard 61-55 ºC touchdown**

Triton-free call rate: **98.5%**

Genotype concordance:[3] **100%**



- Legacy KASP M.Mix (Batch A)
- Legacy KASP M.Mix (Batch B)
- Triton-free KASP M.Mix
- Outlying data points

[1] DNA concentration shown as percentage of neat DNA extraction.

[2] Mean GC% taken from the allele specific primers

[3] Concordance was calculated by comparing the genotypes that were assigned using the legacy KASP Master Mixes versus the genotypes that were assigned using the Triton-free KASP Master Mix.

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Validation Results – Example B

KASP genotyping using sbeadex purified template DNA

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Starting material: **Sunflower Seed**

DNA purification: **sbeadex**

Working DNA concentration: **8.1 ng/µL**

Genotyping format: **Array Tape**

Reaction volume: **0.8 µL**

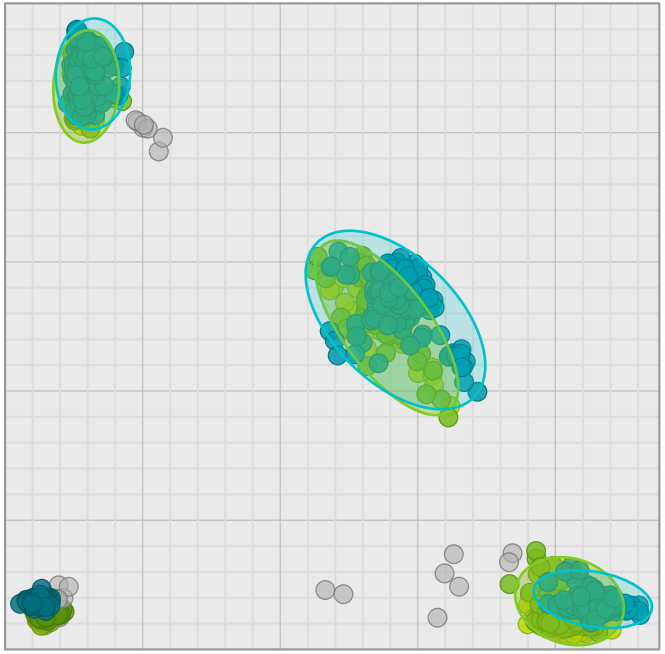
Master mix tested: **KASP v5**

Primer GC content:[1] **40.0%**

Thermal cycling: **65-57 ºC touchdown**

Triton-free call rate: **96.8%**

Genotype concordance:[2] **100%**



- Legacy KASP M.Mix (Batch A)
- Legacy KASP M.Mix (Batch B)
- Triton-free KASP M.Mix
- Outlying data points

[1] Mean GC% taken from the allele specific primers

[2] Concordance was calculated by comparing the genotypes that were assigned using the legacy KASP Master Mixes versus the genotypes that were assigned using the Triton-free KASP Master Mix.

Validation Results – Example C

KASP genotyping requiring optimised cycling conditions

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Starting material: **Whole Blood**

DNA purification: **Kleargene**

Working DNA concentration: **1.0 ng/µL**

Genotyping format: **384-well plates**

Reaction volume: **3.6 µL**

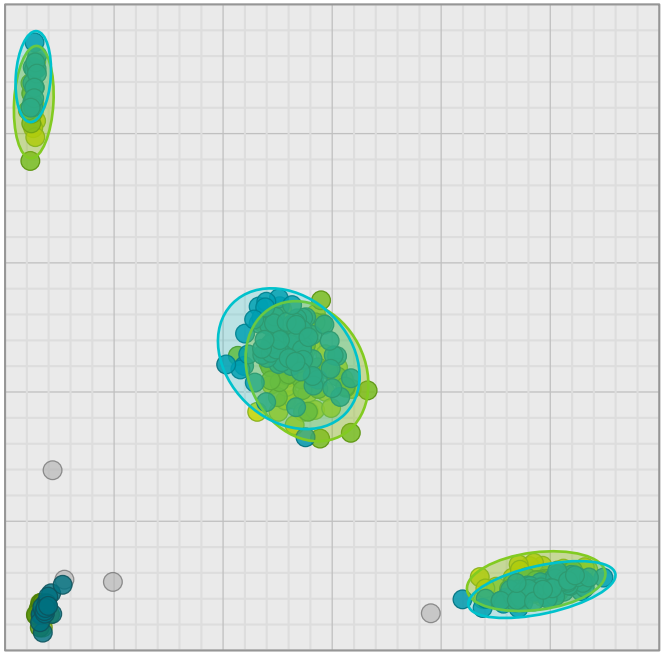
Master mix tested: **KASP v4 (96/384-well)**

Primer GC content:[1] **27.8%**

Thermal cycling: **2-step 57 °C protocol**

Triton-free call rate: **98.3%**

Genotype concordance:[2] **100%**



- Legacy KASP M.Mix (Batch A)
- Legacy KASP M.Mix (Batch B)
- Triton-free KASP M.Mix
- Outlying data points

[1] Mean GC% taken from the allele specific primers

[2] Concordance was calculated by comparing the genotypes that were assigned using the legacy KASP Master Mixes versus the genotypes that were assigned using the Triton-free KASP Master Mix.

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Example of validation results

Completed validation experiments performed using KASP v4 in 384-well and 1536-well plates

Starting material	DNA isolation chemistry	Cluster-plot assessment	Call rate assessment	Genotype concordance assessment	NTC signal assessment
Wheat seed [1]	**sbeadex**	✓ **8 plates passed** (6 required)	✓ **8 plates passed** (6 required)	✓ **8 plates passed** (6 required)	✓ **8 plates passed** (6 required)
Sunflower seed	**sbeadex**	✓ **16 plates passed** (14 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)
Tomato seed	**sbeadex**	✓ **15 plates passed** (14 required)	✓ **14 plates passed** (12 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)
Tomato seed	**QuickExtract**	✓ **16 plates passed** (14 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)
Maize seed [2]	**Hot Shot**	✓ **8 plates passed** (7 required)	✓ **8 plates passed** (6 required)	✓ **8 plates passed** (6 required)	✓ **8 plates passed** (6 required)
Lettuce leaf	**sbeadex**	✓ **16 plates passed** (14 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)
Lettuce leaf	**QuickExtract**	✓ **16 plates passed** (14 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)	✓ **16 plates passed** (12 required)
Whole blood (Human)	**Kleargene**	✓ **32 plates passed** (30 required)	✓ **32 plates passed** (30 required)	✓ **32 plates passed** (30 required)	✓ **32 plates passed** (30 required)
Whole blood (Human) [3]	**Kleargene**	✓ **31 plates passed** (24 required)	✓ **31 plates passed** (24 required)	✓ **31 plates passed** (24 required)	✓ **31 plates passed** (24 required)
Total		**158 plates passed**	**157** plates passed	**159** plates passed	**159** plates passed

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.

[3] These experiments used KASP assay mixes that require **optimised thermal cycling protocols**

Summary

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- Essential change to the formulation of KASP Master Mix
  - Replace the detergent Triton
- Performed extensive validation of the new Triton-free formulations
  - Representative range of species and tissue types
  - Over 850 reaction plates (384-well, 1536-well and Array Tapes)
  - Over 580,000 data points analysed
- Data generated clearly demonstrate the new Triton-free mix has equivalent performance to current formulations

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The change to Triton-free formulations has no impact on the data generated.

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Appendices

Additional data and information

Experimental details

DNA, dispensing and thermal cycling

Robust experimental setup

- Same DNA, same plate, same equipment

DNA diluted 10-100 x as determined for best performance

- In 10 mM Tris-HCl pH 8.3

Reaction set up

	DNA volume	DNA dried down?	Reaction volume
384-well plates	1.5 µL	Yes	3.6 µL
1536-well plates	1.5 µL	Yes	1.0 µL
Array Tape	0.8 µL	Yes	0.8 µL

- Thermal cycling protocols
  - Standard 61-55 ºC touchdown protocol

Protocol stage	Temperature	Duration	Number of cycles
1	94 ºC	15 min	x 1
2	94 ºC 61 ºC	20 seconds 60 seconds	x 10
3	94 ºC 55 ºC	20 seconds 60 seconds	x 26

- Optimised thermal cycling protocols
  - 68-62 ºC touchdown protocol
  - 2-step 57 ºC protocol

Additional reformulated products

RepliKator wash

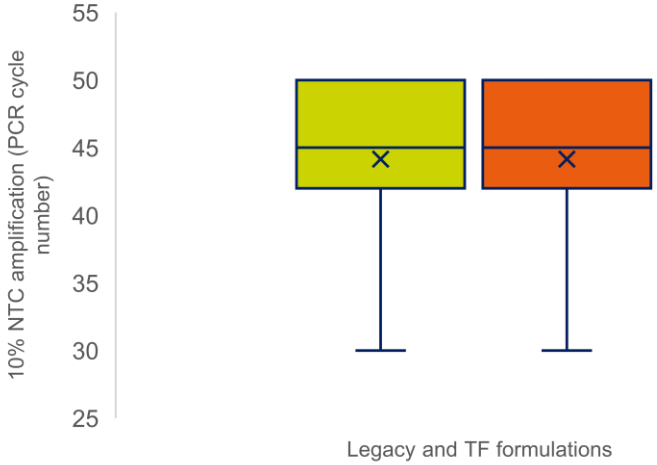
RepliKator wash is used to decontaminate RepliKator tips

Ineffective washing will result in contamination

- Leads to early PCR amplification of NTCs with KASP and BHQ

Comparison of PCR samples prepared with new TF RepliKator wash and current RepliKator wash

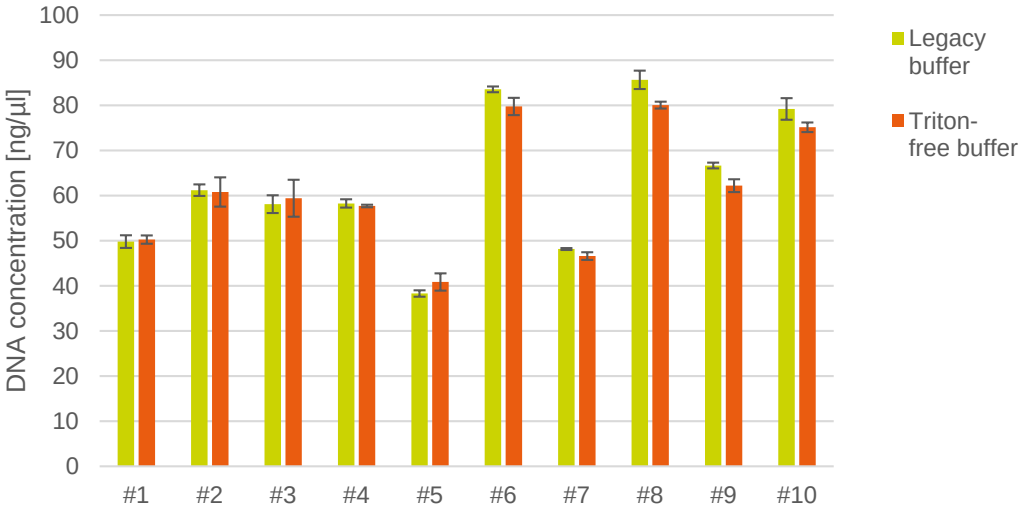
No differences observed



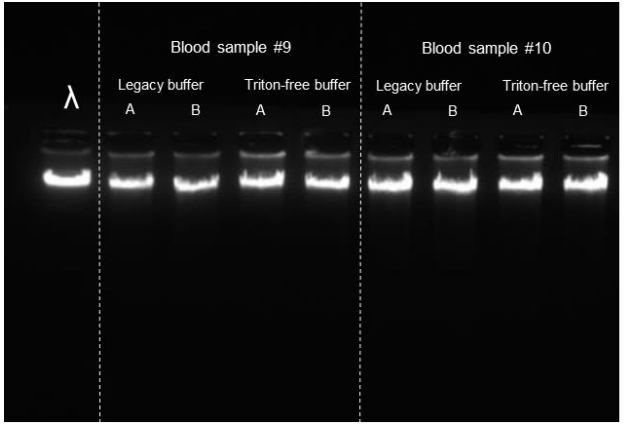
	Legacy formulation	TF formulation
Mean Cycle number at which 10% NTC amplified	44	44
Standard deviation	6.56	6.48

Additional reformulated products

Kleargene



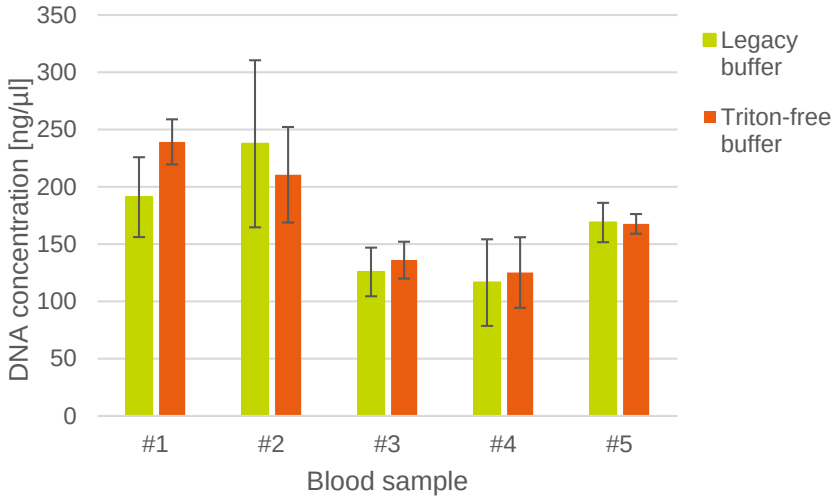
Concentration of DNA extracted from 500 µL blood using Kleargene chemistry with both the legacy buffer and the Triton-free formulation. No significant difference in concentration was observed across the ten samples tested. Samples #1-5 were stabilised in CPD, samples #6-10 were stabilised in EDTA.



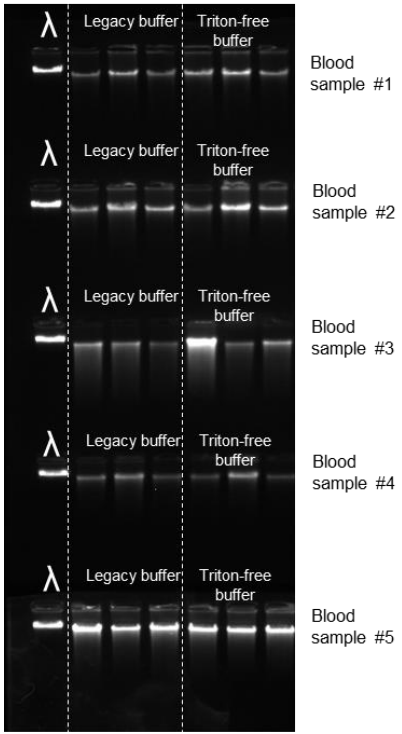
Gel eletropherogram demonstrating integrity of DNA extracted from blood using Kleargene chemistry with both the legacy buffer and the Triton-free formulation. No differences in DNA integrity were observed.

Additional reformulated products

Magbeads



Concentration of DNA extracted from blood using the Mag maxi PLUS kit with both the legacy buffer and the Triton-free formulation. No significant difference in concentration was observed across the five samples tested.



Gel eletropherogram demonstrating integrity of DNA extracted from blood using the Mag maxi PLUS kit with both the legacy buffer and the Triton-free formulation. No differences in DNA integrity were observed.

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Thank you

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