

New Isohelix™ Saliva-Mag DNA Isolation Kit provides a fast and reliable method for sample purification

The new Isohelix™ GeneFix™ Saliva-Mag DNA Isolation Kit (GSM) has been designed and optimised for the extraction of intact genomic DNA of high yield and purity from saliva. Using new high-binding capacity magnetic bead technology, DNA can be purified quickly and cleanly from saliva samples. In combination with GeneFix™ DNA Saliva Collection kits and the GeneFix™ Mag-Rack, Isohelix™ provides a complete solution to sample preparation for demanding downstream analysis.

Methods & Materials

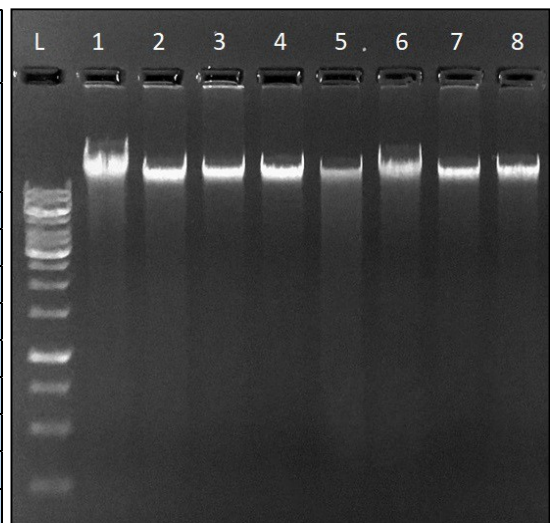
Eight GFX-02 GeneFix™ saliva samples were collected from multiple donors, approximately 2ml of saliva collected per sample. Samples were then treated with 40µl Proteinase K solution (20mg/ml) and were incubated at 60°C for one hour. Following this, collected samples were purified using the Saliva-Mag extraction kit and Mag-Rack, following instructions detailed in the kit protocol. Purified samples were eluted into 400µl Tris buffer.

Purified samples were then analysed for yield, purity and quality of DNA by Qubit dsDNA BR assay, Nanodrop and 1.0% agarose gel electrophoresis. Additionally, 1ng of purified DNA from each of the samples were run on a Real-Time PCR (qPCR) reaction using primers for the human ACTB gene, in order to determine suitability for downstream applications.

Results

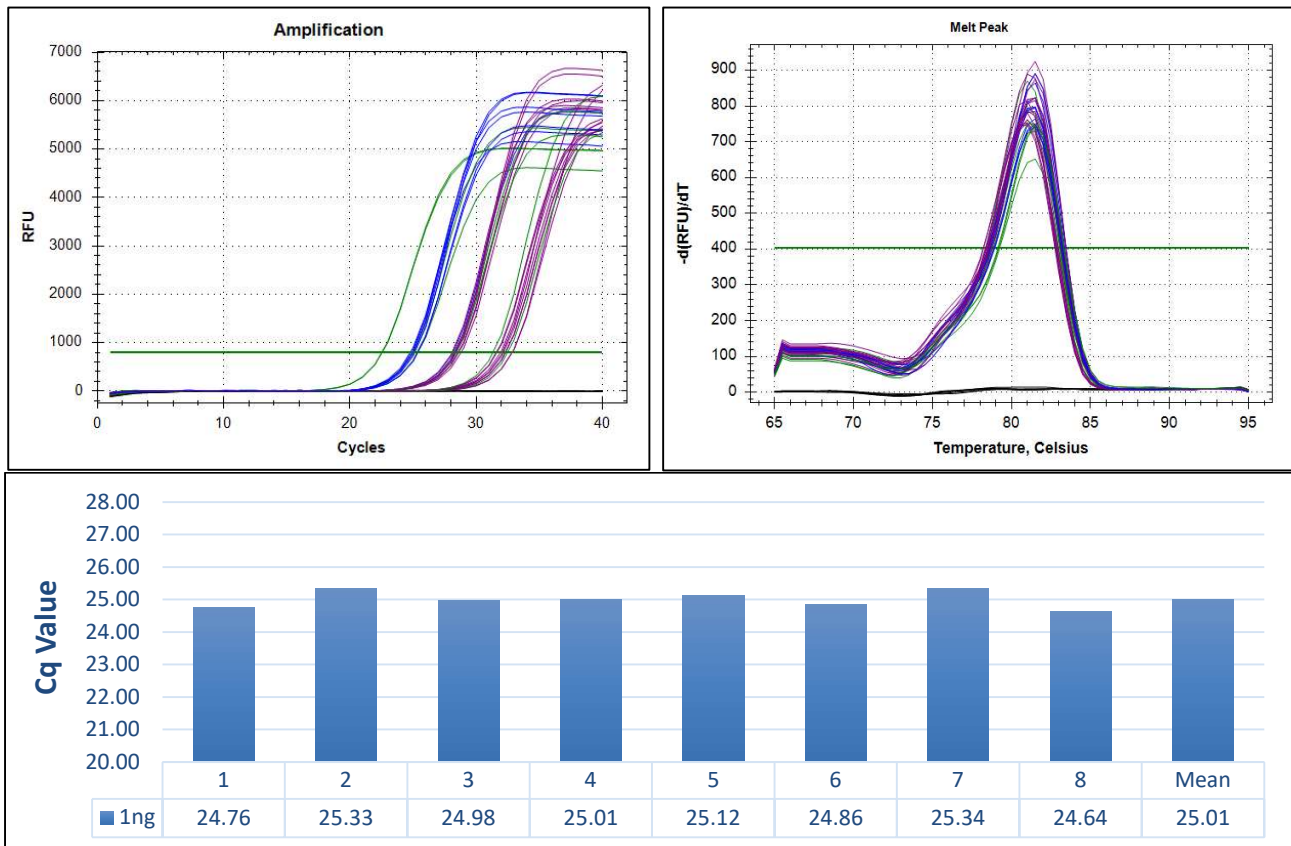
DNA total yield, concentration, purity, and integrity from GFX-02 samples containing 2ml stabilised saliva, extracted using the Saliva-Mag DNA Isolation Kit:

Sample	Qubit dsDNA BR Assay Results		Nanodrop Results	
	Sample Concentration (ng/µl)	dsDNA Total Yield (µg)	A260/280	A260/230
1	141.0	56.40	1.81	1.75
2	73.3	29.32	1.83	1.73
3	43.5	17.40	1.81	1.87
4	44.2	17.68	1.79	1.82
5	49.4	19.76	1.94	1.99
6	117.0	46.80	1.77	1.56
7	45.5	18.20	1.83	1.76
8	63.7	25.48	1.87	2.10
Mean	72.2	28.88	1.83	1.82



DNA isolated from GeneFix™ samples using the Saliva-Mag kit is of high purity, and of yields ideal for most downstream applications. Agarose gel analysis indicates that the samples are intact and of high quality, displaying a single, clean high molecular weight band with minimal degradation.

Amplification of ACTB gene by qPCR show consistent, repeatable results using GeneFix™ Saliva-Mag Kit:



The purified DNA from the saliva samples (blue) were subsequently run on ACTB qPCR alongside a standard curve generated using control human genomic DNA (green) and negative controls (black). In addition to these, sets of tenfold serial dilutions of the samples (purple) were also run to demonstrate the absence of PCR inhibitors in the sample by comparing these to the standard curve controls.

All samples tested generated strong, consistent amplification of the target gene, with low deviation between samples. The subsequent dilutions of samples demonstrate that inhibitors are not present in the reactions, as amplification was in line with the genomic DNA standards. Melt curve analysis of samples display precise and accurate amplification of the ACTB gene.

Conclusions

- The GeneFix™ Saliva-Mag DNA Isolation kit purifies high-quality samples from saliva suitable for downstream applications such as real-time PCR.
- High binding capacity magnetic beads maximise DNA recovery and yield from GeneFix™-collected saliva samples, while preventing co-elution of potential downstream contaminants.
- When used in combination with the GeneFix™ Mag-Rack, up to twelve 4ml GeneFix™ saliva samples can be processed simultaneously, allowing efficient batch extraction of high-yield DNA samples at a faster rate than traditional extraction methods.