The oKtopure tip wash station: decontamination of used tips and problem free re-use with the oKtopure / sbeadex extraction system

Dr. Sarah Holme, Owen McCann;
LGC, Unit 1 - 2 Trident Industrial Estate, Pindar Road, Hoddesdon, Herts EN11 0WZ, UK

The sbeadex™ plant kit has been developed to extract genomic DNA from a wide variety of plant materials (including leaves, seeds and fruits) and can be used for a wide range of plant types.

The coated, magnetic sbeadex particles bind DNA using a novel, two-step binding mechanism in the presence of detergents and salts. After binding and washing steps, the purified DNA is released in the elution buffer.

The oKtopure (KBS-0009-001) provides 'walk-away' automation of the sbeadex DNA extraction process and high-throughput preparation of standardised, high-quality DNA samples. The DNA is suitable for your application whether it be SNP genotyping or high-specification analysis such as next-generation sequencing.

This application note highlights a major cost saving benefit of the oKtopure system when used in combination with its companion piece, the oKtopure wash station (KBS-0009-003).

Cost per extraction is a crucial factor for high-throughput applications. Data from laboratory testing shows how our simple washing protocol using the oKtopure wash station allows complete decontamination of the racked oKtopure tips between extractions, and enables the re-use of oKtopure tips up to 40 times before discarding, saving as much as 50% over alternative platforms.

Figure 1: The oKtopure wash station

Table 1: Specification overview - oKtopure and sbeadex plant

Key features of the oKtopure / sbeadex system	Specification
Elution volume	100 µL
Format / robotic platform	oKtopure
Sample type	Plant samples (leaves, seeds, flour etc.)
Chemistry	sbeadex plant
Final wash	2 binding steps; final wash with pure water
Grade of automation	Full walk-away automation
Nucleic acid purification type	DNA
Average yield sbeadex mini	1 - 15 µg
Average yield sbeadex maxi	20 - 80 µg

oKtopure wash station overview

For sbeadex extractions on the oKtopure, one rack of 96 tips is used per 96-well sample plate for all the DNA pipetting steps of the protocol, except for addition of the three wash buffers and elution buffer (an additional 4 x 96-tip racks which do not come into direct contact with DNA at any time are used). Up to eight sample plates can be extracted at one time on the oKtopure; using a total of 12 x 96-tip racks per 8-plate extraction.

At the end of the extraction process, the 96-tip racks are removed from the oKtopure and washed using the oKtopure wash station to completely remove any residual DNA or buffers. The simple oKtowash™ protocol is summarised below.

The wash station comes supplied with an ultrasonic cleaning bath, 24 x decontamination / clean boxes and 24 x decontamination / clean box tops.

After washing used oKtopure 96-tip racks with the oKtopure wash station and oKtowash protocol, the tips are free from contaminating DNA.

oKtowash protocol overview

- Loading and unloading of the 96-tip racks from the oKtopure is performed using the unload / load new tip function of the software
- The oKtowash supplied must be diluted 1:10 before use in the protocol
- Keeping the tips in their tray, remove the tips from the oKtopure as one unit and place in a decontamination / clean box containing ~200 mL oKtowash (top up as necessary to ensure every part of the tip is submerged) (KBS-0009-002)
- Leave the tips to soak for 30 minutes
- Remove the decontamination / clean box top, shake off excess liquid in the sink and place into the ultrasonic cleaning bath filled with 600 mL of desalted water or desalted water with oKtowash

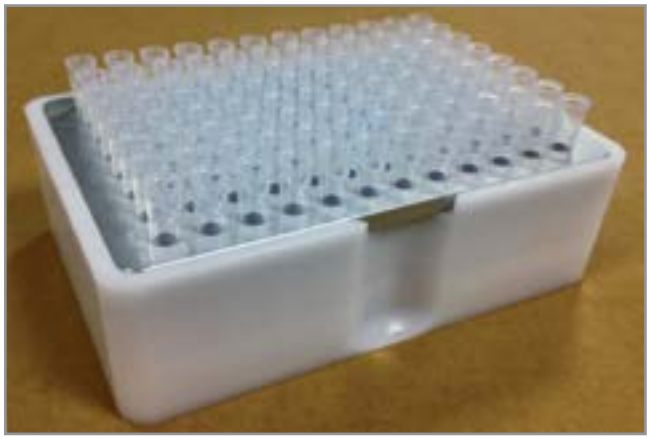


Figure 2: The oKtopure wash station

- Sonicate the tips for 30-60 seconds, until all beads have been washed from both the exterior and interior of the tips (move tips up and down in bath if necessary)
- Remove the decontamination / clean box top, shake off excess liquid as before and place the top into a clean decontamination / clean box filled with approx. 200 mL of fresh, purified water (making sure the box is completely filled up)
- The tips are now fully washed and decontaminated and ready to re-load onto the oKtopure using the unload / load new tip function of the software.

Assessment of oKtowash protocol for decontamination

Full decontamination of any used tips from residual DNA before re-use is crucial to the user.

After washing used oKtopure 96-tip racks with the oKtopure wash station and oKtowash protocol, the tips are free from contaminating DNA. In addition, there is no residual oKtowash on the tips that will destroy DNA when re-used.

A set of 96-tip racks previously used throughout a full sbeadex / oKtopure extraction program for pipetting DNA samples at all stages of the extraction process were put through the oKtowash protocol detailed above. The additional racks of tips previously used only for pipetting clean buffers in the extraction run were also included in the experiment, as ‘no DNA’ control tips.

Detection of any residual DNA from washed tips

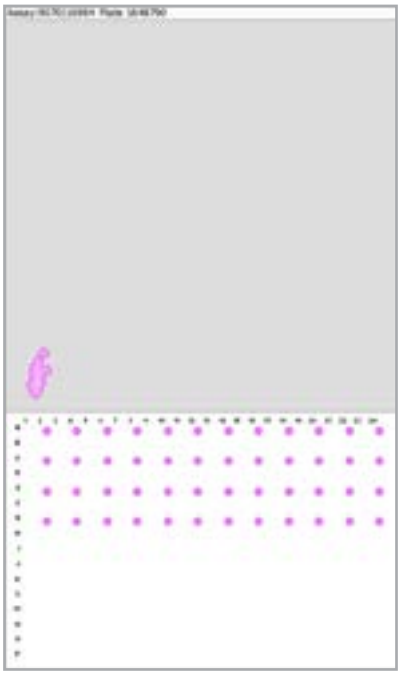
- The 96-tip racks previously used to pipette DNA were washed using the oKtopure washing station and the oKtowash protocol with either oKtowash or water only.
- The washed 96-tip racks were placed into fresh plates containing 100 µL of TE (Tris, EDTA 0.1 mM).
- The TE was aspirated up and down in the tips 80 times; after this 5 µL of the TE was transferred to a fresh 384-well plate.

In order to detect any carryover of residual DNA fragments from the washed tips into the TE, the 5 µL TE sample was then used in one of our a standard QC genotyping assays using our standard KASP chemistry and methods (Figure 3a, b and c).

Detection of any residual oKtowash from washed tips

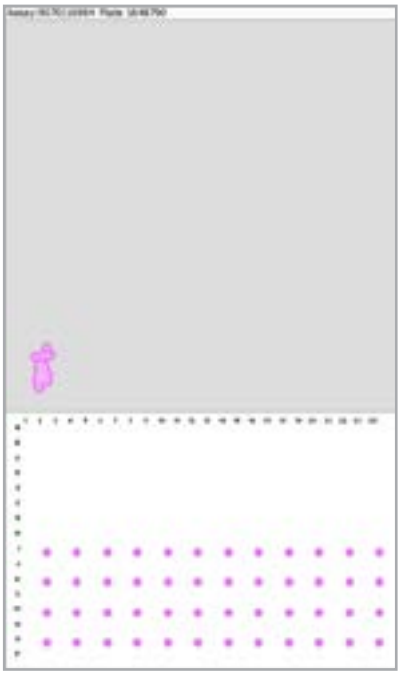
- The 96-tip racks previously used to pipette DNA were washed using the oKtopure washing station and the oKtowash protocol with either oKtowash or water only.
- The washed 96-tip racks were placed into fresh plates containing 100 µL of a working concentration of lettuce genomic DNA.
- The lettuce genomic DNA was aspirated up and down in the tips 80 times before finally transferring 5 µL of the DNA to a fresh 384-well plate.
- The 5 µL of transferred sample was then used for genotyping with a standard QC assay and using our standard KASP chemistry and methods, in order to test for the presence and effect of any potential oKtowash carry over (Figure 4a and b).

Figure 3: Results showing that 96-tip racks used for extracting DNA on the oKtopure are completely free from carryover DNA when washed with the oKtowash protocol using oKtowash, and can be re-used without contamination.



3a. Genotyping data generated with samples dispensed with DNA-free tips (control tips):

No amplification, showing no DNA carryover



3b. Genotyping data generated with samples dispensed using re-used tips washed with oKtowash:

No amplification, showing no DNA carryover

3c .Genotyping data generated with samples dispensed using re-used tips washed with **water only**:

Amplification showing carryover DNA from insufficiently washed tips.

Figure 4: Results showing that 96-tip racks washed with the oKtowash protocol do not transfer any residual oKtowash and can be re-used without causing PCR inhibition.

4a. Genotyping data generated with samples dispensed using re-used tips washed with oKtowash:

Tight clustering of positive amplification signal, showing no inhibition.

4b.Genotyping data generated with samples dispensed using re-used tips washed with **water only**:

Tight clustering of positive amplification signal, showing no inhibition.

Conclusion

After washing used oKtopure tips with the oKtopure wash station and oKtowash protocol, the 96-tip racks are free from contaminating carryover DNA. In addition, there is no residual oKtowash on the tips that will destroy DNA and prevent PCR amplification when the 96-tip racks are re-used.

Appendix: Catalogue information

Catalogue numbers for oKtopure, sbeadex and related products. The kits are available in 960, 2500, 5000, 10000 and 40000 extractions per kit.

Catalogue number	Description	Units
KBS-0009-001	oKtopure high-throughput DNA extraction robot	1
KBS-0009-002	oKtowash, concentrated wash buffer (500 mL)	1
KBS-0009-003	oKtopure off line tip wash option	1
KBS-0009-004	oKtopure mix plates (Thermo 1.2 mL deep well plate)	1
KBS-0009-005	Wash buffer bulk reservoirs (pack of 4)	1
KBS-0009-999	Extended 12 month on-site fully inclusive service contract	1
NAP41610	sbeadex mini plant	960 tests*
NAP41620	sbeadex maxi plant	960 tests*

(*) sbeadex plant kit is also available in different formats for higher throughput customers, please contact our customer service team.

www.lgcgenomics.com

Germany
Ostendstr. 25 • TGS Haus 8
12459 Berlin • Germany

Tel: +49 (0)30 5304 2200
Fax: +49 (0)30 5304 2201
Email: info.de@lgcgenomics.com

United Kingdom
Unit 1-2 Trident Industrial Estate • Pindar Road
Hoddesdon • Herts • EN11 0WZ • UK

Tel: +44 (0)1992 470 757
Fax: +44 (0)1438 900 670
Email: info.uk@lgcgenomics.com

United States
100 Cummings Center • Suite 420H
Beverly • MA 01915 • USA

Tel: +1 (978) 232 9430
Fax: +1 (978) 232 9435
Email: info.us@lgcgenomics.com

3799/LB/0214