

Rapid and reproducible DNA isolation from 1 ml of whole blood with Thermo Scientific KingFisher Flex

*Sini Suomalainen, Maija Partanen, Ritva Javanainen, Virpi Puro and Arja Lamberg
Thermo Fisher Scientific Oy, Vantaa, Finland*



Abstract

Sample preparation is often a limiting step for genomics and proteomics studies. Rapid and efficient isolation of nucleic acids, proteins and cells from complex biological matrixes is needed to get high quality starting material for various experiments. Thermo Scientific KingFisher, a magnetic bead based, automated purification system that provides a quick and easy solution to achieve high quality and reproducible results in purification of nucleic acids, proteins and cells with minimal hands-on time. This technology is based on magnetic rods which move particles through the various purification phases – binding, mixing, washing and elution. The KingFisher® is an open and flexible

system, allowing the user to choose any available magnetic particle based purification kit suitable for the application.

Introduction

The latest member of the KingFisher instrument family, KingFisher Flex, is truly a flexible solution for different types of sample processing needs. KingFisher Flex offers expanded processing volumes for various applications. With the new 24-rod configuration the processing volume can be raised up to 5 ml and with the 96-rod configuration it is possible to achieve the highest throughput in working volumes of 20-1000 µl.

This application note shows the benefits of KingFisher Flex by using genomic DNA isolation from blood as an example.

- The KingFisher technology is based on moving magnetic particles instead of liquids. Reducing the liquid handling, the reagent carryover from one step of the protocol to the next is minimized. The cross-contamination data shows the reliability of the technology.

- The KingFisher is an open platform magnetic particle processor and it allows the user to choose any available magnetic particle kit on the market. Here we present results of isolating gDNA from blood with three different magnetic particle kits.

- The KingFisher is an easy and fast way to purify biological molecules. This poster presents a comparison of gDNA isolation process using magnetic particles with KingFisher Flex and spin column based isolation method.

Materials and Methods

The gDNA isolation was done for the cross-contamination test from 20 ml whole blood pool by using 1 ml of blood for each positive sample well. 1 ml of TE buffer (pH 8.0) was used as negative samples. The test was done by using InviMag Blood Mini Kit / KF96 (Invitex, Germany) and KingFisher Flex 24 format. The positive and negative samples were pipetted to every other well of the 24-well plate (figure 2b). The DNA extraction was done according to InviMag kit instructions for KingFisher by increasing the volumes up 5-fold except using 500 µl Elution Buffer in the elution step. The DNA cross-contamination between wells was tested by performing 50 µl PCR for all eluates of the plate with CD19 primers that produce 720 bp PCR product from human chromosome 16. The PCR products were run on Agilent 2100 Bioanalyzer (Agilent, USA) according to manufacturer's instructions.

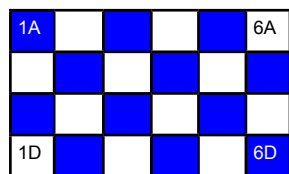


Figure 1. KingFisher Flex 24 deep well plate and the sample orientation on the plate

The three kits used for the DNA isolation kit comparison test were InviMag Blood Mini Kit / 96, AGOWA sbeadex® Blood Kit and BioSprint 96 DNA Blood Kit (Qiagen, Germany). Blood

samples were pooled to 20 ml and 6 samples of 1 ml from the pool were taken to DNA isolation with KingFisher Flex 24 format according to the 5-fold volumes of each manufacturer's instructions. The elution volume was 500 µl for all samples. The absorbances of 260, 280 and 320 nm were measured from 1:5 dilutions of the eluates in a spectrophotometer and the quantity of DNA was calculated from the A260-A320 value. The DNA quality was controlled by the $(A260-A320)/(A280-A320)$ ratio and 5 µl of the samples were run on 1% agarose gel with 100 V. The spectra from 230 nm to 320 nm were measured with Thermo Scientific Varioskan.

The comparison of the DNA isolation methods was done by using InviMag Blood Mini Kit / 96 with KingFisher Flex and QIAamp DNA Blood Midi Kit (Qiagen, Germany). The starting material for both isolation methods was 1 ml of pooled whole blood and the experiment was done according to manufacturer's instructions. With the InviMag kit, 5-fold volumes were used for all the reagents. The DNA quantification and the quality check was done as described in the previous paragraph. The time used for both isolation methods was calculated for hands on and hands free time.

Results

In the cross-contamination test the gDNA yield was in average of 75.0 ng/µl with the ratio of OD 1.8 $[(A260-A320)/(A280-A320)]$. The PCR results from the cross-contamination test show that all the negative samples from every other well (figure 1) produce no PCR product meaning there hasn't been any cross-contamination from the neighbour well. For the magnetic particle kit com-

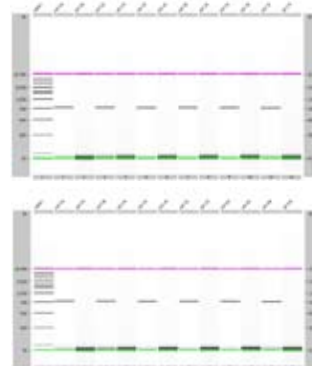
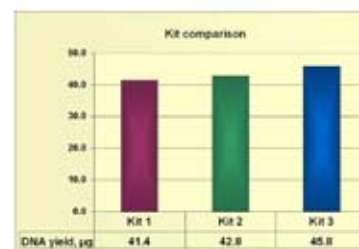


Figure 2. PCR results of the cross-contamination test

parison the results between kits were at similar level. All the kits produced good quality DNA with the OD ratio A260/A280 of 1.8-1.9. The average total quantity of DNA isolated from 1 ml of blood was over 40 µg with all the kits used (figure 3a) and the agarose gel picture showed that the gDNA was intact (figure 3b). The figure 4 shows the absor-

3a.



3b.

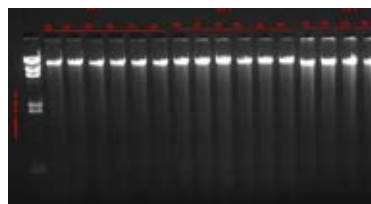


Figure 3. Magnetic particle kit comparison. a) Total yield of DNA (µg) from 1 ml of Blood isolated with three different kits using KingFisher Flex24 b) 1% agarose gel picture of genomic DNA isolates

bance spectra of two DNA eluates compared to pure commercial calf thymus DNA.

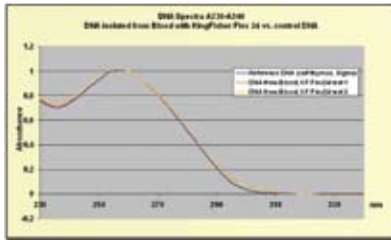
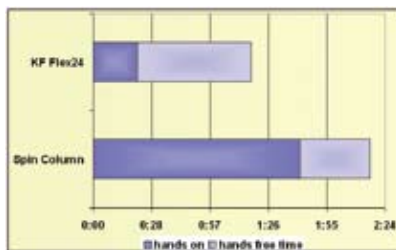


Figure 4. A230-A320 spectra of two DNA isolates extracted by using KingFisher Flex24

In the isolation method comparison both methods used in the experiment produced equal amount of gDNA (figure 5b). The isolation of 24 blood samples with KingFisher Flex instrument took totally 1 hour 18 min and filling the plates (hands on) took 22 min of that time. For the spin column method the total time was 2 hours 17 min with hands on time of 1 hour 42 min (figure 5a).

Discussion

5a.



5b.

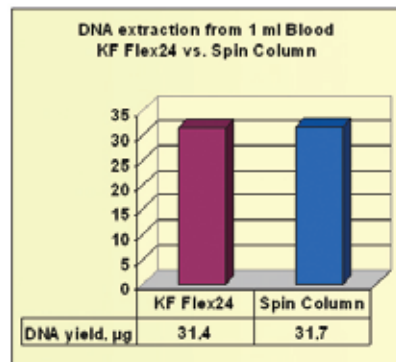


Figure 5. Isolation method comparison. a) The time used for isolating gDNA from 24 blood samples. Comparison between KingFisher Flex24 and spin column based DNA extraction method b) Comparison of the total DNA yield isolated from 1 ml of blood with different methods

These experiments show that KingFisher Flex24 is an excellent tool for high volume purification with different magnetic particle kits. KingFisher Flex is truly open platform that produces high quality reproducible results with all the tested magnetic particle based kits and the results are comparable to spin column method. With the Thermo Scientific BindIt Software it is easy to design protocols for magnetic particle kits from different kit manufacturers.

The data in this poster is based on isolating genomic DNA from blood. The amount of DNA is depending on the amount of the white blood cells in the sample, therefore the blood samples are pooled for kit comparison experiments to minimize the variation due to the starting material.

Conclusions

- KingFisher Flex utilizes fast and reproducible automated method to purify DNA from high volume blood samples
- From 1 ml of blood the total DNA yield is $>40 \mu\text{g}$ with all the kits used
- KingFisher Flex is the only open platform that can be used with different magnetic particle kits resulting high quality DNA with all the kits
- KingFisher Flex is minimizing hands-on work in the laboratory and therefore it saves time and increases productivity
- KingFisher Flex is using unique patented technology which is ideal for wide variety of samples

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

North America:
USA/Canada
+1 800-522-7763

Europe:
Austria
+43 1 801 40 0
Belgium
+32 2 482 30 30
Finland
+358 9 329 100
France
+33 2 2803 2000
Germany national toll free
08001-536 376
Germany international
+49 6184 90 6940
Italy
+39 02 95059 1
Netherlands
+31 76 571 4440
Russia/CIS
+7 095 225 11 15
Spain/Portugal
+34 93 223 3154
Switzerland
+41 44 454 12 12,
UK/Ireland
+44 870 609 9203

Asia:
China
+86 21 6865 4588 or
+86 10 5850 3588
India
+91 22 5542 9494
Japan
+81 45 453 9220
Other Asian countries
+852 2885 4613

Countries not listed:
+49 6184 90 6940
or +33 2 2803 2000

www.thermo.com/kingfisher

© 2008 Thermo Fisher Scientific Inc. All rights reserved. AGOWA speedex is a trademark of LGC Limited. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.