Technical Note:
TN-KF-DWPLATE-0310

High yield and quality with Thermo Scientific Microtiter Deep Well 96 plates in KingFisher Flex process

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The Thermo Scientific KingFisher Flex magnetic particle processor is specifically designed to automate the time-consuming sample preparation of proteins, nucleic acids and cells in 96-well plate formats. We have developed an optimized deep well plate for KingFisher® Flex to enhance the sample preparation and purification process. In this application note, data regarding magnetic particle collection efficiency and RNA isolation using the Thermo Scientific Microtiter Deep Well 96 plate is analyzed.

Introduction

KingFisher Flex uses an advanced patented technology in which magnetic rods move particles through the processing steps to provide efficient and reproducible purification of proteins, nucleic acids and cells from a variety of starting materials. The target molecules proceed automatically through binding and washing steps until the final purified material is eluted from particles for use in a variety of downstream applications. KingFisher Flex 96-well format is compatible with PCR plates, KingFisher 96 plates and deep well plates providing a wide volume processing range from 20 to 1000 µl. To enhance the purification process, we have an optimized 96 deep well plate for KingFisher Flex called Microtiter Deep Well (DW) 96 plate. In this application note we describe the benefits of the Microtiter DW 96 plate with KingFisher Flex 96-well format and the clear advantage of using Microtiter DW 96 plates for the magnetic particle collection efficiency and total RNA isolation, as an example. The data has been generated by using the previous model of KingFisher Flex, KingFisher 96.

The Microtiter DW 96 plate is specifically designed according to the shape of the KingFisher Flex 96 tip comb for deep well magnets and the KingFisher Flex 96 (KF and deep well) heating blocks. All liquid volumes and tip movements are optimized for the Microtiter DW 96 and the bottom height of the plate. The use of other types of 96 deep well plates may cause unexpected mixing issues due to the divergent well volume and the bottom height of the plate. If heating is used in the KingFisher Flex protocol, the heating block will rise to the processing position at the bottom of the plate. The shape of the Microtiter

DW 96 plate is designed to reach a maximal efficiency of heating. If other types of plates are used, the heating block might raise the bottom height of the plate and cause unsuitable mixing or even instability of the KingFisher Flex instrument (Figure 1).

Material and methods

Magnetic particle collection efficiency

Magnetic particle collection efficiency was measured using a Microtiter DW 96-well plate and compared with plates of other manufacturers. Four deep well plates were filled with 1 ml of 0.02 % Tween20. 30 µl of Dynabeads M-280 Streptavidin (10 mg/ml, Invitrogen) was added to the defined wells of the 1st plate. After taking the control sample, a KingFisher run was performed. In the run, magnetic particles were mixed, collected, transferred from plate to plate, and finally discarded. Test samples were taken from the wells where particles were processed. Particle residues in the wells were defined using a Beckman Coulter Z2 particle counter. Residue percentage of the particles was calculated by comparing the particle amount in the control sample and in the test sample. Figure 2 shows the comparison of the particle residue percentage between the plates.

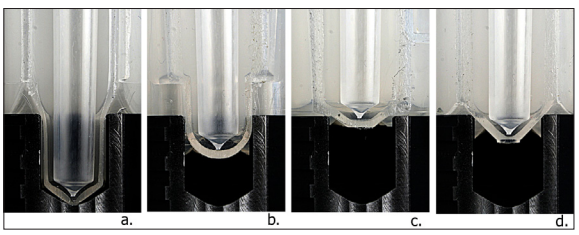

Figure 1. The sectional view of the Microtiter Deep Well 96 plate (1.a) and deep well 96 plates of other manufacturers (1.b, c and d) and the KingFisher Flex 96 deep well heating block

Total RNA isolation

Total RNA was isolated from HEp-2 cells using KingFisher 96 and MagAttract RNA Cell Mini M48 Kit (Qiagen). The isolation from 15 replicate samples (1 million cells per well) was performed using Microtiter DW 96 plate and reference plates from two other manufacturers. The purified RNA was eluted in KingFisher 96 plate; otherwise the samples were processed in deep well plates (e.g. incubation and washing steps). The purified RNA samples were analyzed with Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip kit (Agilent Technologies). Comparison of the average RNA yields are shown in Figure 3. The average relation of ribosomal 28S and 18S RNA is presented in Figure 4. In Figure 5, a typical electropherogram of total RNA sample isolated using Microtiter DW 96 plate is shown.

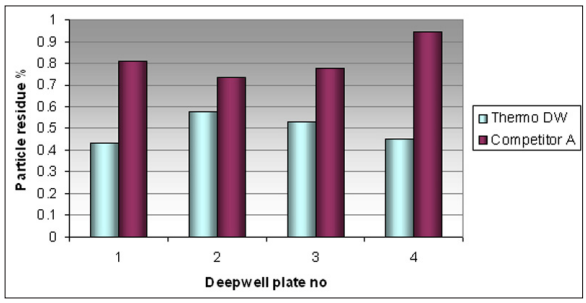

Figure 2. Magnetic particle collection efficiency defined using Beckman Coulter Z2 particle counter. With Microtiter DW 96 plate the collection efficiency is more than 99.4%

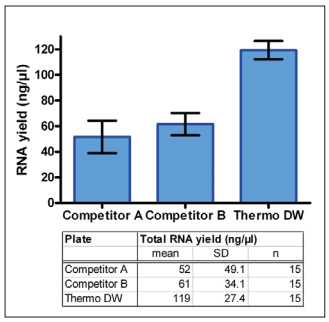

Plate	Total RNA yield (ng/µl)		
	mean	SD	n
Competitor A	52	49.1	15
Competitor B	61	34.1	15
Thermo DW	119	27.4	15

Figure 3. Comparison of total RNA average yields and standard deviations between Microtiter DW 96 plate and two other plates. Analysis is performed using Agilent Bioanalyzer 2100

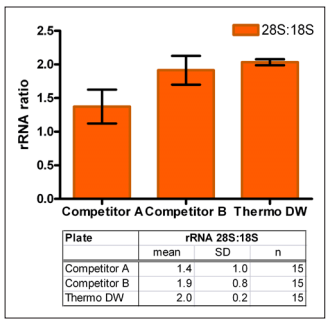

Plate	rRNA 28S:18S		
	mean	SD	n
Competitor A	1.4	1.0	15
Competitor B	1.9	0.8	15
Thermo DW	2.0	0.2	15

Figure 4. Comparison of ribosomal 28S and 18S RNA ratio. Ratio 2:1 (28S:18S) is a good indication that RNA is completely intact. (Agilent Bioanalyzer 2100)

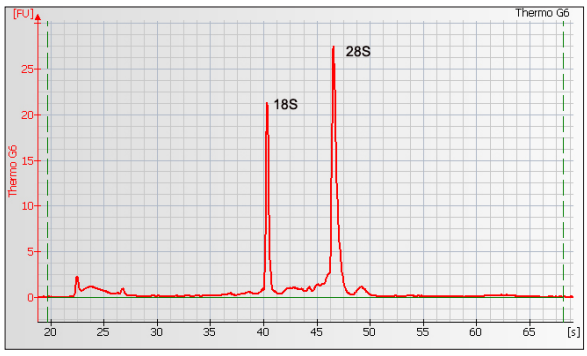

Figure 5. Electropherogram of total RNA sample isolated using Microtiter DW 96 plate. The 18S and 28S peaks are clearly visible indicating high quality and intact total RNA. (Agilent Bioanalyzer 2100)

Summary of results

Magnetic particles are collected more efficiently from the Microtiter DW 96 plate than from the plate of another manufacturer. In practice more than 99.4 % of the particles were transferred from well to well during the run when Microtiter DW 96 plates were used (see Figure 2).

In total RNA isolation the RNA yield and quality were clearly improved when Microtiter DW 96 plates were used in the KingFisher purification process. The average RNA yield obtained using Microtiter DW 96 plate was 119 ng/µl, which is significantly higher when compared to the yields obtained using plates of other manufacturers (see Figure 3). In addition, the standard deviation of the calculated yields was lower with samples processed in Microtiter DW 96 plates, indicating a uniform purification process.

The ribosomal RNA (rRNA) ratio between 28S and 18S was found to be 2:1 in average when Microtiter DW 96 plate was used in the purification process. In Figure 4, rRNA average ratios and standard deviations between Microtiter DW 96 plate and two other plates are compared. An electropherogram of a certain RNA sample isolated using Microtiter DW 96 plate is shown in Figure 5. As a conclusion, overall Agilent Bioanalyzer results clearly indicate that RNA isolated with Microtiter DW 96 plate is of high quality and completely intact.

Conclusions

The data presented in this application note clearly shows that optimal extraction results are achieved when Microtiter DW 96 plate is used as a part of the KingFisher 96-well process. Due to the optimized well bottom design, the liquid and particle movement in the well is highly efficient. As a conclusion, it is highly recommended to use Microtiter DW 96 plates in all KingFisher 96-well purification processes to achieve the best performance.

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