

High yield and quality with new Thermo deep well plates in KingFisher® 96 process



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

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Introduction

KingFisher 96 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 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 96 is compatible with PCR plates, KingFisher 96 plates and deep well plates though providing a wide volume processing range from 20 to 1000 μ l. To further enhance the purification pro-

cess, Thermo has developed an optimized 96 deep well plate for KingFisher 96 called Thermo Microtiter DW plate. These new Thermo deep well plates can only be used with KingFisher Software 2.6 or 2.6.2. In this technical note we describe the clear advantage of the new plate using magnetic particle collection efficiency and total RNA isolation as an example.

Materials And Methods

Magnetic particle collection efficiency

Magnetic particle collection efficiency was measured using Thermo Microtiter DW well plate and plate from another manufacturer as a reference. Four deep well plates were filled with 1 ml of 0.02% Tween20. 30 μ l 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 96 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. The particle residues in the well were defined using Beckman Coulter Z2 particle counter. The residue percent of the particles was calculated by comparing the particle amount in the control sample and in the test sample. In figure 1 the particle residue percent between plates is compared.

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 Thermo DW plate or with 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, washing steps). The purified RNA samples were analyzed on Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip kit (Agilent Technologies). In figure 2, comparison of average RNA yields are shown. In figure 3, the average relation of ribosomal 28S and 18S RNA is presented. In Figure 4, a typical electropherogram of total RNA sample isolated using Thermo DW plate is shown.

Summary of results

Magnetic particles are collected from Thermo DW plate more efficiently when compared to the plate from other manufacturer. In practice, more than 99,4 % of the particles were transferred from well to well during the run when Thermo DW plates was used (see figure 1).

In total RNA isolation the RNA yield and quality was clearly improved when Thermo DW plates were used in KingFisher 96 purification process. The average RNA yield obtained using Thermo DW plate was 119 ng/µl being significantly higher when compared

to yields obtained using other plates (see figure 2). In addition, the standard deviation of the calculated yields was lower with samples processed in Thermo DW plates indicating even purification process. The ribosomal RNA (rRNA) ratio between 28S and 18S was found to be 2:1 in average when Thermo DW plate was used in purification process. See results in figure 3 where rRNA average ratios and standard deviations between Thermo DW plate and two other plates are compared. See also figure 4, where an electropherogram of certain RNA sample isolated using Thermo DW plate is shown. As a conclusion, overall Agilent Bioanalyzer results clearly indicates that RNA isolated with Thermo DW plate is high quality and completely intact.

Conclusion

The data shown in this technical note clearly shows that improved and even results are achieved when Thermo DW plate is used as a part of the KingFisher 96 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 Thermo DW plates and KingFisher Software 2.6.2 in all KingFisher 96 purification processes to achieve the best performance.

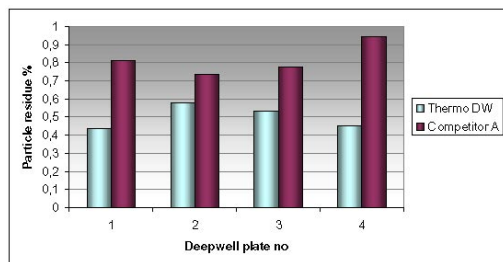


Figure 1. Magnetic particle collection efficiency defined using Beckman Coulter Z2 particle counter. With Thermo Microtiter DW plate the collection efficiency is more than 99.4%.

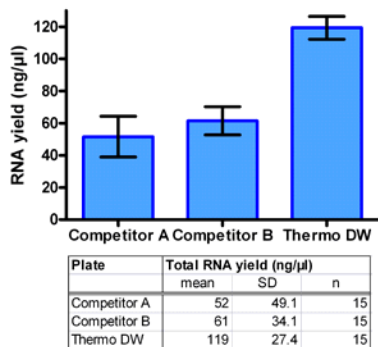


Figure 2. Comparison of total RNA average yields and standard deviations between Thermo Microtiter DW plate and two other plates. Analysis performed using Agilent Bioanalyzer 2100 (Agilent Technologies).

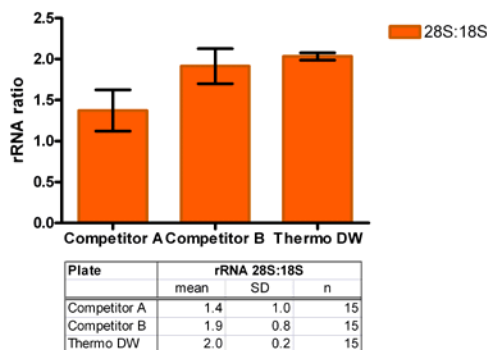


Figure 3. Comparison of ribosomal 28S and 18S RNA ratio. Ratio 2:1 (28S:18S) is a good indication that RNA is completely intact. (Agilent Bioanalyzer 2100, Agilent Technologies).

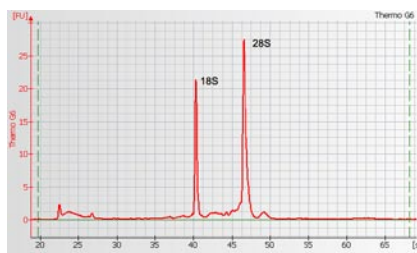


Figure 4. Electropherogram of total RNA sample isolated using Thermo Microtiter DW plate. The 18S and 28S peaks are clearly visible indicating high quality, intact total RNA. (Agilent Bioanalyzer 2100, Agilent Technologies).

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