KingFisher - A Multifunctional Tool for Proteomics

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Introduction

In the proteomics area with the increasing capacity of the mass spectrometers, sample preparation is becoming a limiting step for protein identification. Magnetic particle based technology provides a rapid and easy solution for automation of the sample preparation step.

KingFisher technology which utilizes magnetic beads and rods offers easy to use automation for purifying several hundreds samples per day. The unique patented technology is based on concept where magnetic particles are transferred instead of liquids (Fig. 1). KingFisher magnetic particle processors offer flexible automation for customers having different needs for throughput. The processing range is from 20µl to 1000µl which enables elution in small volumes and results in highly concentrated samples. The KingFisher platform offers ultimate flexibility since it allows running different applications in one platform, for example DNA, RNA, cell and protein purification.

Results

KingFisher system can be used for various protein applications (Table 1). It has been successfully utilized for peptide fractionation in front of mass spectrometer (Fig. 2 & 6). KingFisher system can also be used for phage display application to automate consecutive laborious selection rounds and minimize manual work. It provides constant reproducibility from assay to assay (Fig. 3 & 4). Automated method for the isolation of recombinant polyhistidine tagged proteins using KingFisher 96 or KingFisher mL has been also developed. Using this system the purification of 96 proteins directly from bacterial lysate takes less than 30 minutes. Automated purification of histidine-tagged-CAT (HAT-CAT) protein from bacterial lysates results in intact proteins with high purity (Fig. 5). The functional proteins can then be directly used in several drug discovery applications.

Conclusion

- KingFisher is easy to use tool for various protein applications
- 96 parallel samples can be purified in less than 30 minutes
- The purification yields with reproducible results
- Washing and bead collection is very efficient
- Fractionation products can be directly used in several drug discovery applications

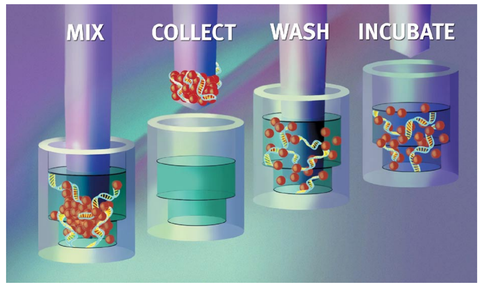


Figure 1. KingFisher technology is based on magnetic rods which move magnetic particles instead of liquids.

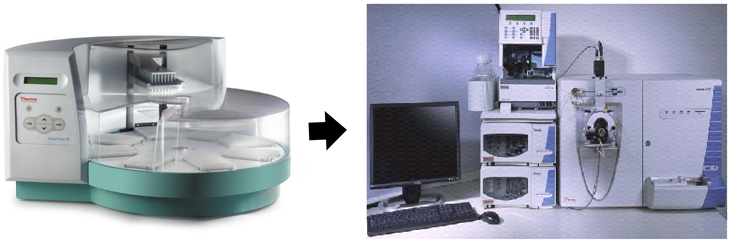


Figure 2. KingFisher 96 is suitable for protein fractionation in front of mass spectrometry.

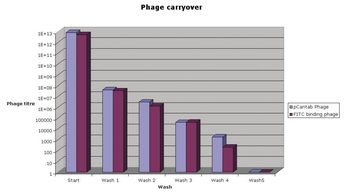


Figure 3. KingFisher mL was used for washing steps in phage display to remove unspecific phages. The unbound phage was completely removed in 5 washes using KingFisher mL.

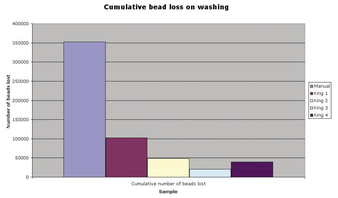
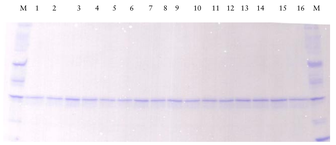


Figure 4. Comparison of the amount of beads lost during manual and KingFisher mL washings. During the KingFisher mL washings the collection of the beads is efficient and bead loss is minimal. Bead loss was shown as the cumulative loss over 7 washes.

Figure 5. Purification of histidine-tagged proteins from bacterial lysates using KingFisher 96.

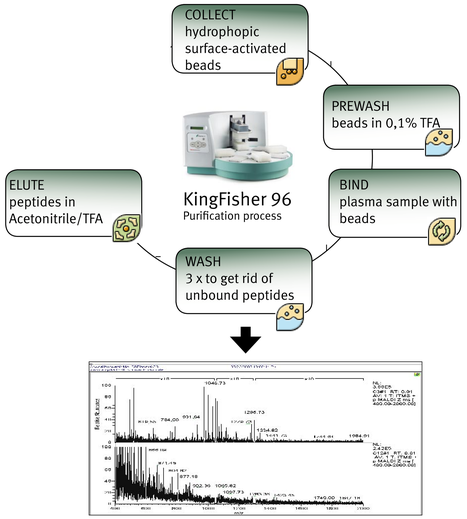


5. Bacterial culture was expressed in 96 deep well plate and used directly in KingFisher purification process. 10 µl sample from different elution wells was analyzed in SDS-page and stained with Coomassie Brilliant Blue. Proteins were eluted in 100 µl of 50 mM imidazole. M = marker. The Technology used for Dynabeads® TALON™ is licensed from BD Biosciences Clontech. BD TALON is a registered trademark of Becton, Dickinson and company

Table 1. Examples of Suitable Protein Applications for KingFisher Platforms

APPLICATION	BEADS	WEB
Protein Fractionation	Dynabeads® WCX , Dynabeads® RPS	www.dynalbiotech.com
Albumin Removal	BioMag® ProMax Albumin Removal Kit	www.bangslabs.com
Immunoglobulin Removal	BioMag® ProMaxIgG Removal Kit	www.bangslabs.com
Targeted Protein Purification with customers antibody	Dynabeads®M-270 Epoxy or M-450 Toxylactivated	www.dynalbiotech.com
Immunoprecipitation	Dynabeads® Protein A, Dynabeads Protein G BioMag® Protein A, BioMag® Protein G	www.dynalbiotech.com www.bangslabs.com
Polyclonal Antibody Purification	Dynabeads® M-280 Sheep anti-Rabbit IgG BioMag® Plus Protein G particle Antibody Isolation Kit BioMag® Goat Anti-Rabbit IgG	www.dynalbiotech.com www.bangslabs.com www.bangslabs.com
Monoclonal Antibody Purification	Dynabeads® Rat and Mouse IgM BioMag® Goat anti-Mouse IgM BioMag® Goat anti-Rat IgM	www.dynalbiotech.com www.bangslabs.com
Recombinant Protein Purification	Dynabeads® TALON™ Ni-NTA Magnetic agarose Beads Strep-Tactin Magnetic beads	www.dynalbiotech.com www.qiagen.com www.qiagen.com
Biotinylated Protein Isolation	BioMag® Plus Streptavidin Particle Biotin Binding starter kit	www.bangslabs.com

Figure 6. Plasma peptide profiling with KingFisher 96



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