Streamlining Nucleic Acid and Protein Isolations with Exclusion-based Sample Preparation (ESP)

Mary C. Regier[1], Scott M. Berry[2], David John Guckenberger Jr[2], Karen M. Kleman[1], and David J. Beebe[2]

[1]Gilson Inc., Middleton WI, USA, [2]Salus Discovery LLC, Madison WI USA

Introduction

Exclusion-based sample preparation (ESP™) is a new technique for isolating nucleic acids, proteins, cells, and viruses from biological samples. Unlike conventional "bind-wash-elute" magnetic bead methods, ESP streamlines the isolation process by using surface tension and hydrophobicity to exclude any unbound contaminants from paramagnetic particle (PMP)-bound biomolecules. By simply passing PMP-bound biomolecules through aqueous / air interfaces, analytes-of-interest are purified rapidly and efficiently. Here, we adapt the ESP "SLIDE" configuration into a compact EXTRACTMAN™ device, whereby a hydrophobic strip positioned on a sliding head transfers the bead-bound material from crude cellular extracts, through one or more wash buffers, and into an elution buffer in a matter of a seconds by simply moving magnets positioned above the strips and below a customized microtiter plate in sequence. Both manual and automated versions of EXTRACTMAN were used to rapidly isolate mRNA, DNA, and proteins from crude extracts using an assortment of commercial kits and reagents (e.g., Life Technologies Dynabeads®, Promega MagneSil®, Pierce Protein A/G PMPs, Beckman Coulter Agencourt). In each case, the EXTRACTMAN performed equivalently or better on metrics of recovery and purity of nucleic acids compared to traditional tube-based methods. In addition, EXTRACTMAN required significantly less hands-on time and fewer liquid handling steps. Selective enrichment and high recovery of recombinant proteins were also observed with the gentle and rapid exclusion method of EXTRACTMAN, which promotes weak binding associations and retention of proteins with fast off-rates.

How does the ESP Technology work?

Conventional magnetic bead capture methods for extracting target molecules from biological samples involve multiple "add and remove" liquid transfer steps (Fig. 1A). With ESP technology (Fig. 1B), the PMPs are moved instead of the fluid; target biomolecules are enriched, contaminants are left behind, and samples are conserved.

A. Conventional magnetic bead capture methods

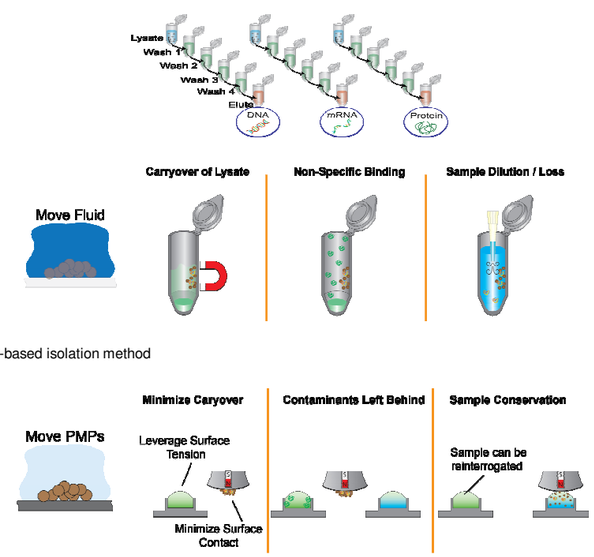

Figure 1. By moving paramagnetic particle (PMP)-bound analyte (B), rather than moving fluid (A), ESP is able to obtain high analyte purity in fewer steps than conventional protocols.

ESP Technology inside EXTRACTMAN™

The EXTRACTMAN is based on the "SLIDE" ESP technology, enabling effortless and effective purification of biomolecules in a matter of seconds.

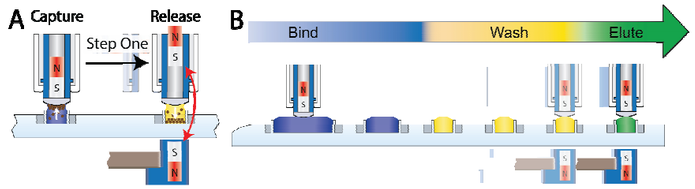

Figure 2. A) ESP uses a hydrophobic surface attached to a magnetic head to transfer PMP-bound analyte with minimal carryover. B) Magnets positioned beneath reagent wells mediate PMP release. Multiple capture-and-release processes are run sequentially to purify analyte.

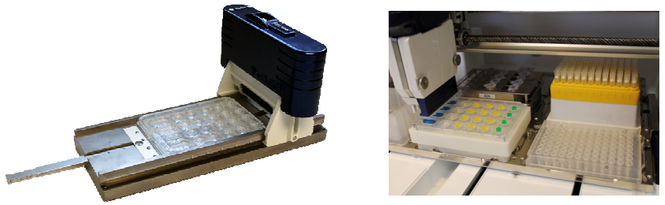

Figure 3. The EXTRACTMAN uses ESP to rapidly isolate four target molecules in parallel. Both manual (left) and automated (right, with PIPETMAX®) versions have been developed.

mRNA Isolations

Yields and purity levels of mRNA samples isolated with EXTRACTMAN were equivalent or better than conventional tube-based methods as evidenced by similar or lower Cq values in qPCR experiments.

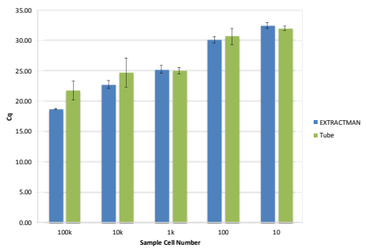

Figure 4. RT-qPCR analysis of mRNA isolations by EXTRACTMAN and tube-based methods. RNA was isolated from eGFP expressing MCF7 cells using Dynabeads® mRNA DIRECT™ Kit from Life Technologies. qPCR was performed with QIAGEN OneStep RT-PCR Kit (n=3). Error bars represent 1 SD of the mean.

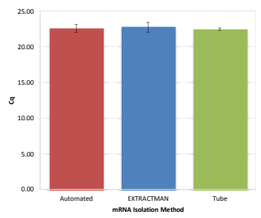

Figure 5. RT-qPCR analysis of mRNA isolations by automated EXTRACTMAN, manual EXTRACTMAN, and tube-based methods. See Fig. 4 legend for more details.

DNA Isolations

The EXTRACTMAN is a universal platform that can be used with a variety of commercial DNA extraction kits

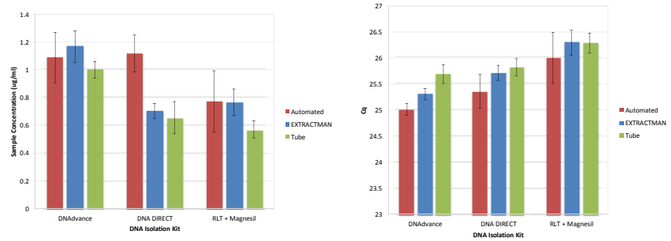

Figure 6. DNA isolation with EXTRACTMAN or conventional manual tube-based methods using various commercial kits. DNA was extracted from 10,000 MCF7 cells using tube/rack setups, manual and automated EXTRACTMAN devices, and quantified by fluorescence (Qubit) or qPCR (n=4). Kit manufacturer protocols were followed with slight modifications. Error bars represent 1 SD of the mean.

Protein Isolations

Recombinant proteins are greatly enriched at high yields and low carry-over using gentle ESP technology

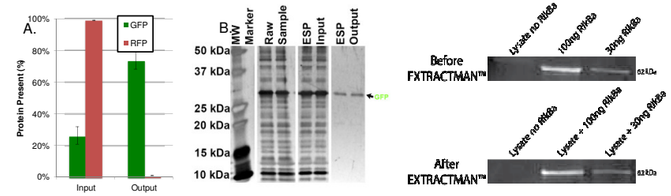

Figure 7. Isolation of minor target protein (GFP) from GFP-RFP expressing E.coli extracts. **A)** GFP (present at ¼ the level of RFP in crude extract) was enriched 77-fold (compared to RFP) with anti-GFP antibody and ESP technology. The green and red fluorescence of the input and output wells were measured with a fluorescent scanner (n=5). **B)** Silver stained gels demonstrate the purity of the target protein (GFP) from non-specific, contaminating proteins.

Figure 8. Western blot analysis of rIKBa protein spiked into mammalian cell lysate before and after immunoprecipitation with anti-IKBa antibody, Protein G beads, and EXTRACTMAN. Greater than 96% of the spiked-in recombinant protein was recovered among the abundant contaminating proteins in the cell lysate. Protein levels were quantified with a fluorescent scanner.

Summary

- **ESP differs from conventional tube-based capture methods by moving the magnetic bead-analyte complex instead of the wash and elute buffers.**
- **ESP-based EXTRACTMAN uses surface tension and hydrophobic properties to gently and rapidly pull PMP-analyte complexes through liquids (and air) in a series of input, wash, and output wells, enriching the sample and excluding unbound contaminants in a matter of seconds.**
- **Yield and purity levels of mRNA and DNA samples isolated with manual or automated EXTRACTMAN are equivalent or better than conventional tube-based methods.**
- **Rapid isolation of target proteins with high specificity and recovery is achievable with EXTRACTMAN.**

References

Casavant BP, Guckenberger DJ, Jr., Beebe DJ, and Berry SM. 2014. Efficient Sample Preparation from Complex Biological Samples Using a Sliding Lid for Immobilized Droplet Extractions, *Anal. Chem*. 86, 6355–6362.

Goel S, Chin E, Fakhraldeen S, Berry S, Beebe D, and Alexander C. 2012. Both LRP5 and LRP6 Receptors Are Required to Respond to Physiological Wnt Ligands in Mammary Epithelial Cells and Fibroblasts, *JBC* 287(20), 16454–16466.