# EXTRACTMAX™: AUTOMATED MAGNETIC BEAD PURIFICATION OF WEAKLY-BOUND PROTEIN-PROTEIN INTERACTIONS

## APPLICATION NOTE AN1057

### BENEFITS

- Fully automated, multi-sample, affinity-based purification
- Gentle magnetic bead purification preserving weak protein-protein interactions
- Disposable bead capture strips allow for continuous sample processing with no cross-contamination

### ADDRESSED ISSUES

- Gilson bead capture strips and microplates allow for multiple wash steps before elution without subjecting samples to harsh shear forces
- Specially designed magnetic EXTRACTMAX head allows for four samples to be run in parallel
- Nine position EXTRACTMAX bed allows for integration of magnetic bead purification into larger complex protocols

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## INTRODUCTION

The use of magnetic beads, also known as magbeads, is a common technique employed in the purification of proteins during biological sample preparation. These beads are typically composed of paramagnetic particles with surfaces functionalized with various epitopes designed to bind to DNA and proteins of interest.[1] Compared to standard co-immunoprecipitation (Co-IP) beads, the use of paramagnetic particles allows for the beads to be affixed using a magnet and washed without the need to subject them to the intense G-forces of centrifugation. However, traditional magbead extractions usually involve using a micropipette to wash the sample. Aspiration and dispensing using a micropipette can subject samples to shear forces that could disrupt weaker, transient protein-protein interactions.[2]

Gilson's exclusion-based sample preparation (ESP™) technology is a gentler and more rapid alternative to traditional Co-IP methods that takes advantage of the paramagnetic qualities of the beads as well as the surface tension of the wash and elution liquid to

**Figure 1**
EXTRACTMAX™

gently pull the beads from one well to another and efficiently exclude unbound material from transferring from wash step to wash step.[3] Until recently, this ESP technology was only available through EXTRACTMAN® and was still a manual process. Gilson's EXTRACTMAX is a new integration of EXTRACTMAN's ESP technology combined with Gilson's automated liquid handler, PIPETMAX® 268, to create a fully automated platform for gentle magnetic bead-based purification and

isolation of target compounds that could potentially be missed when using other Co-IP methods (Figure 1).

Gilson's specially designed EXTRACTMAX microplates (Figure 2) allow for up to four washes and a final elution step. The plates have four rows, and with EXTRACTMAX's magnetic head, up to four samples can be prepared in parallel. This application note demonstrates the use of EXTRACTMAX in moving antibodies across a plate through wash solutions using the EXTRACTMAX magnetic head, running four samples in parallel, and highlighting the minimal protein loss from well to well when using magnetic beads.

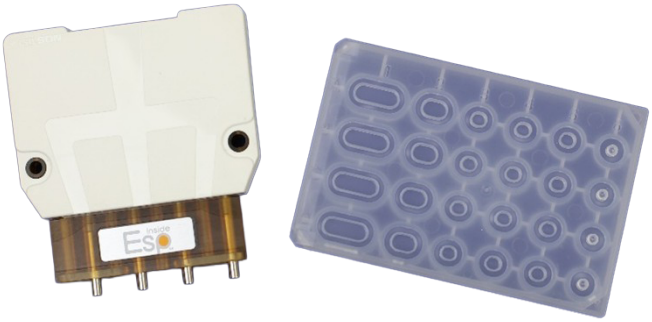


**Figure 2**
EXTRACTMAX magnetic head (left)
EXTRACTMAX extraction microplate (right)

## MATERIALS AND METHODS

Donkey anti-rabbit immunoglobulin antibodies (IgG) were acquired from ImmunoReagents, Inc. All other solutions and reagents, including Pierce™ Protein A/G Magnetic Beads, were sourced from ThermoFisher and used as received. Magnetic bead purification of IgG was performed on a Gilson EXTRACTMAX equipped with a MAX8x200 Pipette Head as well as an EXTRACTMAX magnetic head. Protein quantification of individual wells was performed using a Bradford assay and read using a CLARIOstar plate reader from BMG Labtech.

For preparing EXTRACTMAX, magnetic beads were rinsed and washed according to the manufacturer's instructions. Subsequently, 100 µL of beads were incubated with 100 µL of 1 mg/mL IgG for approximately one hour at room temperature before the experiment. Reservoirs containing phosphate buffered saline (PBS) and Bradford assay reagent were set up on EXTRACTMAX along with disposable tips, a 96-well plate, the EXTRACTMAX extraction microplate, a rack for disposable bead capture strips used by the EXTRACTMAX magnetic head, and Rack Code 424 holding tubes containing IgG, magnetic beads, and a prepared IgG-magbead mixture.

For the method, EXTRACTMAX was programmed to carry out all of the following steps in a single continuous and automated fashion without human intervention.

Using the MAX8x200 Pipette Head, the special EXTRACTMAX microplate's wells were fully filled with PBS, creating a convex meniscus in each well. The first well in the first column contained only PBS. 50 µL of magnetic beads were added to the first well in the second row, and 50 µL of IgG was added to the first well of the third row. These first three rows served as negative controls. Lastly, 100 µL of the IgG/magbead mixture was added to the first well of the bottom row (Figure 3).

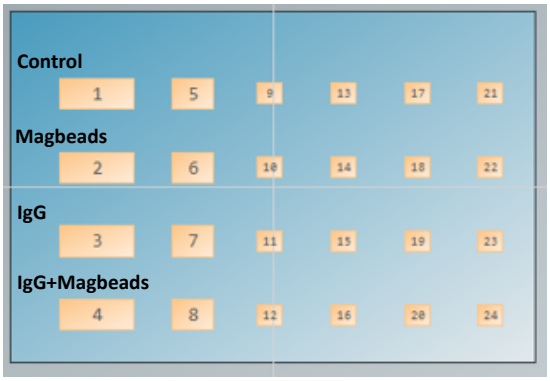



**Figure 3**
EXTRACTMAX microplate template with rows labeled according to their running conditions. (from top to bottom): Control (no antibody/no beads), magnetic beads only, antibody only, both antibody and magnetic beads.

The EXTRACTMAX magnetic head was affixed with a Gilson bead capture strip and lowered down onto the EXTRACTMAX microplate to contact the solutions in the first column of the microplate wells (Figure 4). Using ESP, the magnetic head slowly moved left to right across the plate, dragging the magnetic beads from one column to the next, stopping at each column and releasing the beads for approximately 30 seconds to allow for mixing and washing of the beads (Figure 5). After reaching the fifth column, the beads were released into the wells.

Using stock IgG, a serial dilution was made to construct a calibration curve in the 96-well plate on EXTRACTMAX. Next, a sampling of predetermined wells was aliquoted into the 96-well plate, and all wells with liquid were filled with Bradford reagent to quantify the protein concentration in each well.

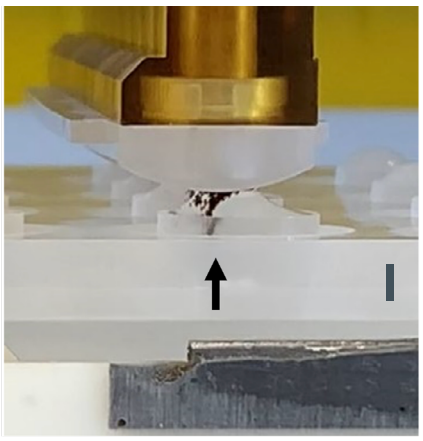


**Figure 4**
Close-up of EXTRACTMAX magnetic head picking up magnetic beads

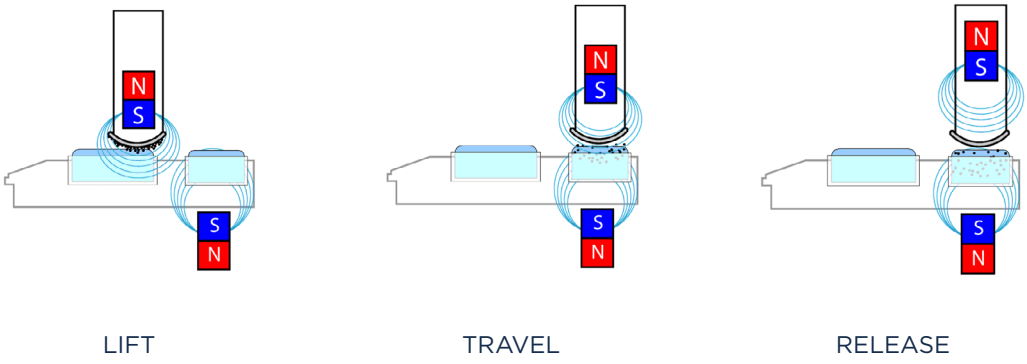



**Figure 5**

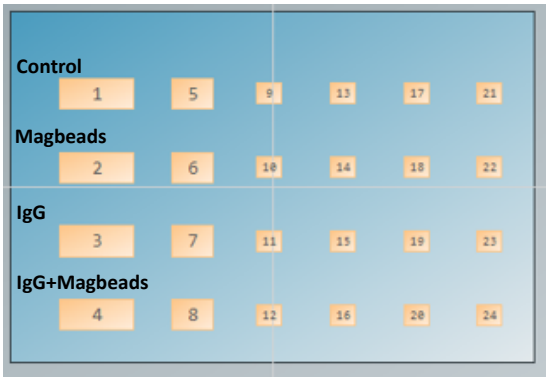
Illustration of magnetic mechanism of ESP™ on EXTRACTMAX. During the release step, the magnets inside the EXTRACTMAX magnetic head are pulled up, releasing the beads into the well. This process is repeated, moving the beads from well to well until the beads are sufficiently washed and deposited into the final well.

This entire process was performed twice with a different selection of aliquots taken from each EXTRACTMAX microplate. The first run examined various points across all rows to show the movement of IgG with the magnetic beads across the plate in relation to the other rows. The second run examined each well in the row that contained both IgG and magbeads to assess sample loss with each subsequent wash step. All data points were collected in triplicate. After the Bradford reagent was dispensed into the 96-well plate according to the assay instructions, the plate was incubated at room temperature for approximately ten minutes before being manually transferred to the plate reader, where the absorbance at 595 nm of each well was recorded.

## RESULTS AND DISCUSSION

The first experiment examined protein concentration at various points across the entire EXTRACTMAX microplate. Aliquots were taken from the first, third, and fifth wells of each row (Figure 6). After the addition of the Bradford reagent, it was visibly apparent that there was a high concentration of protein that remained in the first wells of the IgG only row and IgG+magbeads row (rows 3 and 4, respectively). While not as intensely blue, there was still a significant amount of protein in the last wells of the IgG+magbeads row (well 20). There was also a lesser but still significant amount of protein detected by the Bradford assay in the last well of the IgG only row (well 19). Because the magnetic particles are functionalized with proteins, it is not surprising to see a signal in the last wells of that row. Quantifying the absorbance of the wells with the plate reader showed that very few, if any, magbeads remained in the first well and that most, if not all, made it to the final well in its row. The absorbance reading in the last well of the IgG+magbeads row (well 20) was significantly higher than the corresponding magbeads only row, (well 18) showing that there was IgG binding to the magbeads and they were able to be taken up, washed, and deposited to the far side of the microplate. The high protein concentrations in well 4 suggest that there was an excess of IgG to saturate the magbeads and still have enough remaining in the starting well to elicit a strong signal from the Bradford assay. Another observation to highlight is that although the magbeads were dragged across the microplate with only surface tension keeping the magbeads in solution, there is no cross-contamination between the different rows along the microplate.

The second experiment examined the protein concentration of each well in the IgG+magbeads row to check for any sample loss between the start and end of the EXTRACTMAX microplate (Figure 7). After the addition of Bradford reagent, it was visibly apparent that the first and fifth wells contained significant protein (wells 4 and 20), corroborating the results from the previous experiment. Quantification of the protein concentration of the middle wells (wells 8, 12, and 16) showed no significant presence of any protein, indicating that there was no real loss of protein from any of the wash steps.



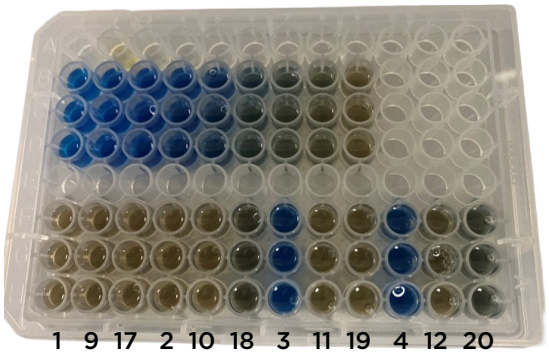
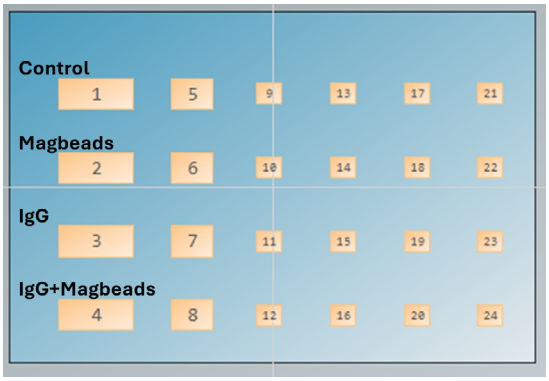




**Figure 6**

Illustration of EXTRACTMAX microplate with rows and wells labeled for reference. (left)

Bradford assay of IgG calibration curve and selected wells. Rows B-D contain IgG in decreasing concentrations in triplicate. Rows F-H are triplicate aliquots of the specified wells labeled below. (right)



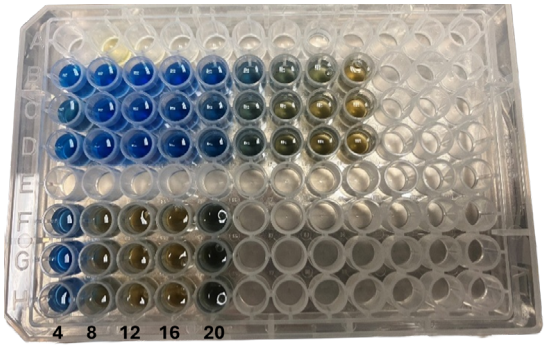




**Figure 7**

Illustration of EXTRACTMAX microplate with rows and wells labeled for reference. (left)

Bradford assay of IgG calibration curve and selected wells. Rows B-D contain IgG in decreasing concentrations in triplicate. Rows F-H are triplicate aliquots of the specified wells labeled below. (right)

## CONCLUSIONS AND BENEFITS

Magbead purification and extraction of proteins has become a staple technique across biological labs to collect, analyze, and study various protein-protein interactions; however, the use of traditional micropipettes to wash and elute targets of interest can still introduce shear forces capable of disrupting weakly bound compounds. These compounds are then lost, and potentially critical binding interactions can go undiscovered. The ESP technology present in Gilson's EXTRACTMAX allows for even gentler magbead extraction, preserving these transiently bound interactions intact, with the added benefit of increasing throughput by enabling the extraction of multiple samples in parallel.

By incorporating the ESP technology from EXTRACTMAN into the existing framework of PIPETMAX 268, EXTRACTMAX can perform gentle magbead extractions and pulldowns in an automated fashion, freeing up a lab technician's time and energy. Furthermore, not only does EXTRACTMAX allow for automated magbead extractions, but it can also process up to four samples in parallel, uses disposable strips and tips to eliminate cross-contamination risks, and the large EXTRACTMAX bed allows for running more complicated automated protocols in which a magbead extraction may be just a part of the entire workflow.

## REFERENCES

1. Berensmeier S. Magnetic particles for the separation and purification of nucleic acids. Appl Microbiol Biotechnol. 73(3):495-504.
2. Berry SM, Chin EN, Jackson SS, Strotman LN, Goel M, Thompson NE, Alexander CM, Miyamoto S, Burgess RR, Beebe DJ. Weak protein-protein interactions revealed by immiscible filtration assisted by surface tension. Anal Biochem. 15:447:133-40.
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