Application Note

Microlute™ Protein Precipitation Plate

Summary

Isolation of high quality, protein-free samples is an vital prerequisite for successful mass spectrometry (MS) analysis. Protein is usually precipitated by salt induced or isoelectric precipitation. Porvair Sciences offer an alternative 96 well filtration plate for protein precipitation. The high throughput format can be advantageous compared to traditional centrifugation based techniques. Illustrated here in this short application note is the effectiveness of the Microlute™ Protein Precipitation Plate for the removal of proteins.

Biological samples commonly contain proteins that interfere with downstream applications.
Pharmaceutical research of small molecules in biological fluids such as plasma or serum requires their removal from the protein matrix before analysis. If proteins are not removed, they can precipitate out into the HPLC mobile phase leading to column or system blockages. With LC-MS, analyte response can be affected by proteins causing ion suppression or enhancement in the source.

The Microlute™ Protein Precipitation Plate (Porvair Sciences, Part number: 240100) is designed to
efficiently precipitate proteins from plasma and serum. The novel dual frit design ensures the sample is held up until ready to use, there is no leakage and there is a consistent flow rate of the sample when vacuum is applied. To demonstrate the performance of the Microlute™ Protein Precipitation Plate a protein crash with plasma, using solvent precipitation, was compared against a leading competitor.

Method

A protein crash was performed using 100µl pig plasma (Sigma P2891) and protocol followed according to manufacturers' instructions. In brief, each precipitation plate was loaded onto a Porvair Sciences 96 well collection plate (Product code: 219250). Acetonitrile was added in 3:1 ratio with pig plasma, agitated for ~1-3 minutes on a plate shaker followed by centrifugation at 500 x g for 3 minutes to collect the eluent. The eluents were dried down using Porvair Sciences Ultravap™ Levante using a 96 straight needle head (Product codes: 229226 & 229036 resp) at 30°C with 4 psi nitrogen flow for 35 minutes at needle heights 17mm to 41mm. The samples were then reconstituted in 100 ml PBS and tested for protein content using the BIORAD DC™ Protein assay (Product code: 5000111). Samples were run in triplicate.

Results

The Microlute™ Protein Precipitation Plate presented with clear filtrates and showed no evidence of protein breakthrough. This is highlighted in the significantly lower amounts of protein in the eluent compared to the competitor (Figure 1). The Microlute™ Protein Precipitation Plate also has much greater well-to well reproducibility.

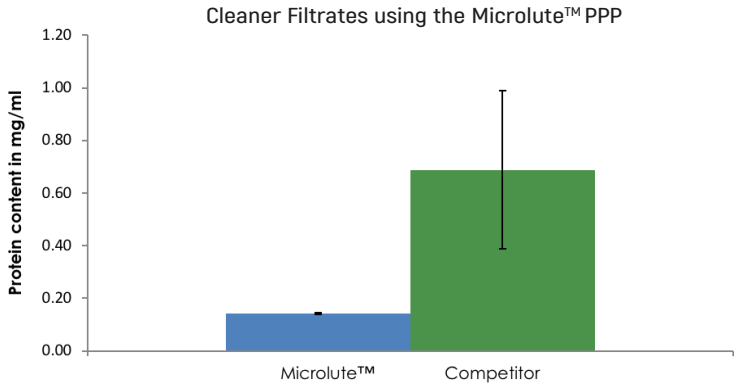

Figure 1. Protein content of filtrates from Microlute™ Protein Precipitation Plate measured using BIORAD DC™ Protein Assay.

Conclusion

The Microlute™ Protein Precipitation Plate offers a simple and cost effective solution for protein precipitation. This short application note highlights significantly reduced proteins from plasma in a high throughput format allowing downstream applications for HPLC or LCMS. The novel dual frit is low bind so will maximise recovery of the analytes/compounds. With no leakage, blockage efficient precipitation and being easily automatable the Microlute™ Protein Precipitation Plate is the ideal choice for high throughput protein precipitation.

Find out more at www.microplates.com/microlute

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