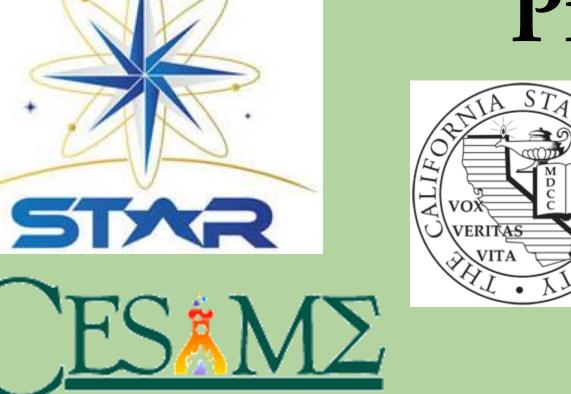
Proteomic Sample Preparation Method: Rodent Blood High Abundant Protein Removal





Introduction

proteomic biomarkers in blood plasma and blood

serum is the broad dynamic range of its protein

constituents. For example, 70-95% of all the rat

plasma proteins are comprised of albumin,

Therefore, a successful system of proteomic

developed successful ways to remove these

abundant proteins is needed to examine lower

abundant proteins of interest. Researchers have

proteins from human blood samples, but many

high abundant protein removal kits for mouse

and rat models vary in the efficiency of actual

targeted protein content that is removed. In

addition, there are different systems for high

abundant protein removal, such as antibody

for high abundant protein removal were

serum.

based approaches and newer resin/bead based

constructs. In this study, three different methods

compared on rat blood plasma and mouse blood

Methods

Protea Prep

TCA MWCO

Rodent

MARS

Immunoglobulin (IgG), and transferrin.

sample preparation to remove these high

One of the biggest challenges for the study of

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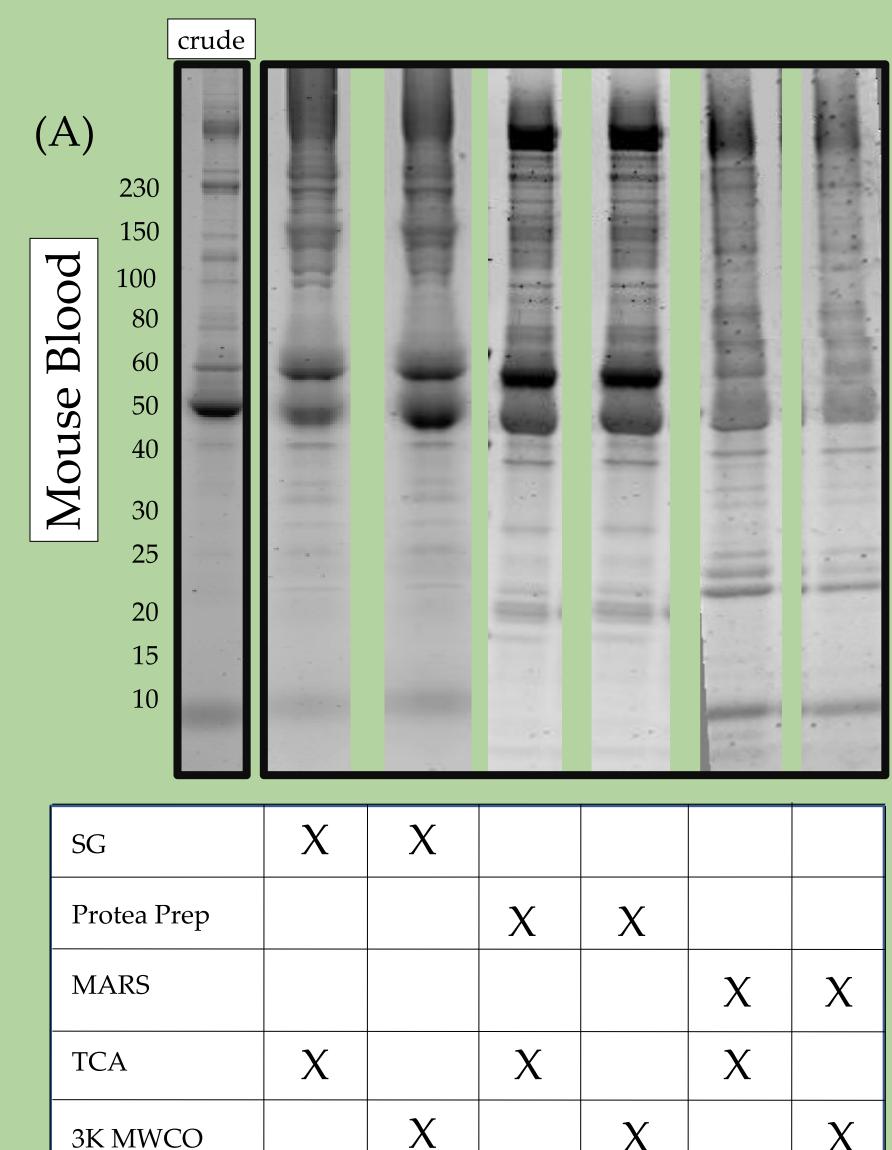
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Results

Table 1: Shows start/end amount of protein for each sample prep method combination, as well as % remaining protein.

	ug of crude starting sample	High abundant protein removal method	Buffer exchange/ method	average remaining protein (ug)	average % remaining protein
Mouse serum	1756.09	SG	TCA	75.50	4.30%
			MWCO	54.24	3.09%
	1062.53	Protea Prep	TCA	64.20	3.66%
			MWCO	15.83	0.90%
	1756.09	MARS	TCA	80.50	4.58%
			MWCO	47.88	2.73%
Rat Plasma	540.42	SG	TCA	50.42	2.87%
			MWCO	37.43	2.13%
	540.42	Protea Prep	TCA	60.47	3.44%
			MWCO	83.66	4.76%
	540.42	MARS	TCA	38.16	2.17%
			MWCO	79.95	4.55%



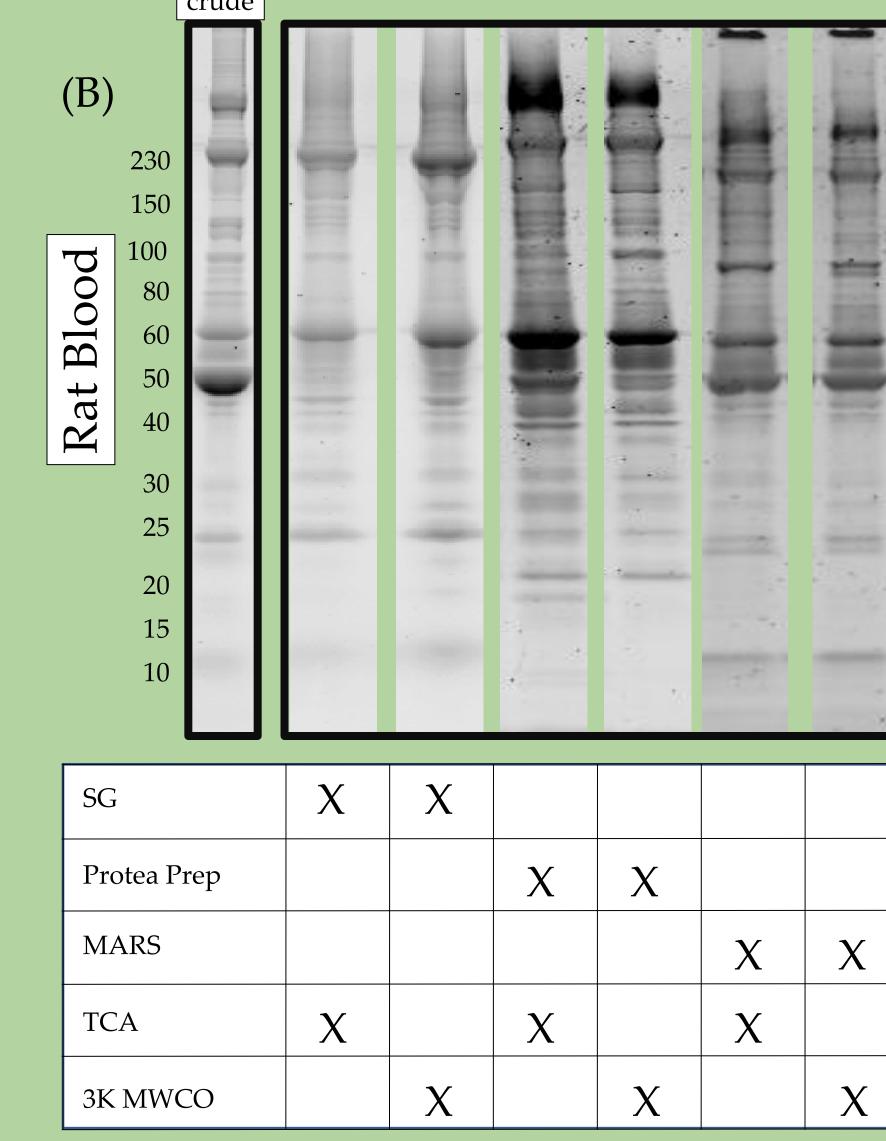


Figure 1: All sample prep combinations were analyzed by SDS-PAGE (Invitrogen 4-20% Tris-Glycine). (A) Shows mouse serum samples. (B) Shows rat plasma samples.

High abundant protein removal methods | Proteins removed Swell Gel blue kit (SG) Albumin Pierce #89845 **Protea Prep** Albumin Protea #SP-200 Multiple affinity removal system (MARS) Albumin, IgG, Agilent #5188-5217 (mouse-3) transferrin

Concentration and buffer exchange methods

Trichloroacetic acid/acetone precipitation (TCA)

3K Molecular weight cut-off (MWCO)

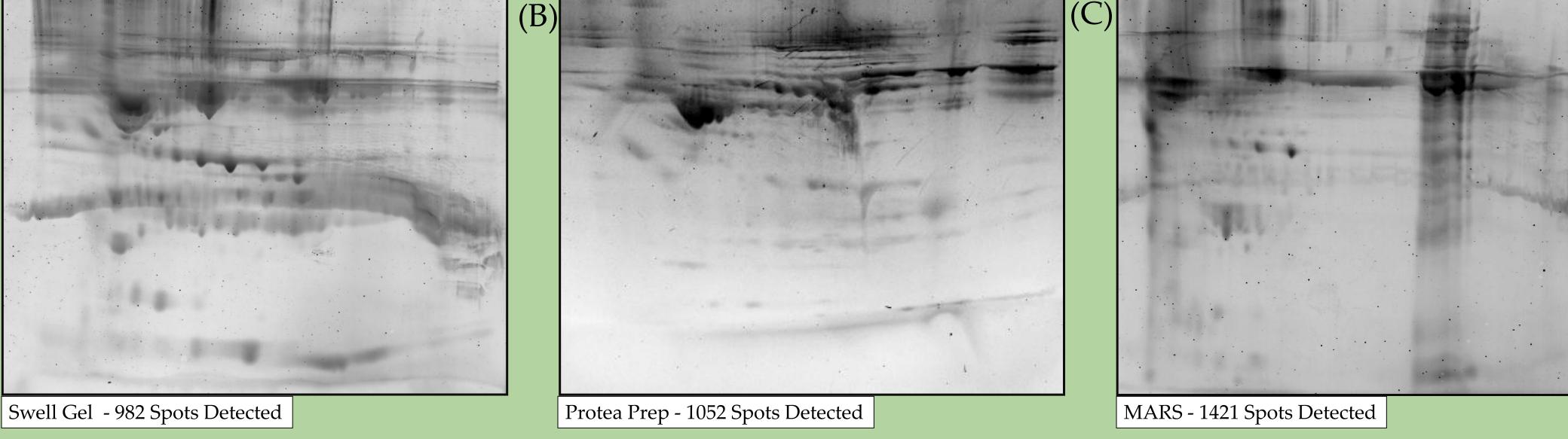


Figure 2: Mouse blood in conjunction with MWCO preparation was selected and used to compare the 3 high abundant protein removal methods, Swell gel (A), Protea kit (B), MARS (C) by 2D gel separation. Spot patterns were evaluated using DeCyder 7.0 and total number of spots detected are noted for each method.







Conclusion

This preliminary study aims to compare methods of high abundant protein removal, as well as concentration/buffer exchange methods. For mouse serum, the best high abundant protein removal method was achieved using the Mouse-3 Multiple affinity removal system. This gave the greatest appearance of lower abundant spots by SDS-PAGE, as well as the greatest number of spots detected on 2D gel. Less efficient albumin removal was seen via the swell gel and Protea Prep methods.

For rat plasma, SG and MARS shows some high abundant protein depletion, but the Protea Prep kit demonstrates the greatest increase in the appearance of lower abundant protein spots by SDS-PAGE.

Trichloroacetic Acid/acetone precipitation and 3K molecular weight cut-off preparations showed little difference.

References

Brett A. Chromy, Arlene D. Gonzales, Julie Perkins, Megan W. Choi, Michele H. Corzett, Brian C. Chang, Christopher H. Corzett, and Sandra L. McCutchen-Maloney. Proteomic Analysis of Human Serum by Two-Dimensional Differential Gel Electrophoresis after Depletion of High-Abundant Proteins Journal of Proteome Research 2004, 3, 1120-1127.

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