Fast and automated protein purification with MACHEREY-NAGEL Protino® 96 Ni-NTA purification system

Purifying proteins is central to both studying their function, structure or interactions as well as for industrial scale manufacturing of bio-therapeutics. The power of modern molecular genetics to provide large quantities of proteins that were previously difficult to obtain has sparked an explosion of interest in practical and theoretical aspects of protein purification.

- ***High well to well reproducibility – easy scale up possibilities***
- ***High capacity and high recovery – low agarose bead costs and therefore low operational costs***
- ***Leak-free incubation without cross-contamination – reliable results without additional purification steps***

Introduction

A common purification technique involves engineering a sequence of 6 to 8 histidine into the N- or C- terminus of the protein backbone.

Expressed His-tagged proteins from crude extracts can easily be purified by immobilized metal ion affinity chromatography (IMAC). The polyhistidine tag strongly binds to nickel ions that are immobilized on a solid support like agarose beads. All non-tagged proteins are washed away.

The protein can be eluted with imidazole, which competes with the polyhistidine tag for binding sites, or by a decrease in pH (typically to 4.5), which decreases the affinity of the tag to the resin.

Figure 1: Microlab STARlet

Method Description

Re-suspension and transfer of the Protino® Ni-NTA Agarose beads into the filter plate with a total bed volume of 50 µl in each well is done by the ML STARlet (Figure 1).

After equilibration of the Protino® Ni-NTA Agarose beads on the vacuum station (Figure 2) clear lysate is transferred to the filter plate which is parked on a shaker. Batch binding of the protein is done at room temperature by shaking the lysate bead mixture at 1100 rpm for 20min.

Liquid filled filter plates can be directly handled on a shaker without carry over or contamination, due to a leak free filter plate. After a washing step which is done on the vacuum station the bound protein is eluted in 2 steps with 200µl of 250mM imidazole each. The eluates are combined and the purity is proven by SDS-PAGE analysis.

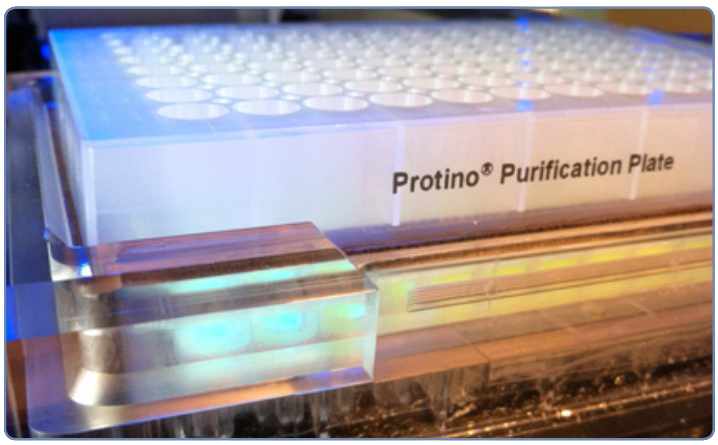

Figure 2: Protino® Purification Plate

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Validation

Using 50 µl of Protino Ni-NTA Agarose yields up to 2.5 mg of target protein per well can be expected, according to the stated binding capacity (50 mg protein per 1/mL bed volume). In this study each well of the Protino® Purification Plate was loaded with increasing amounts of His-tagged protein from 0.6 to 1.6 mg. The incubation of the plate on a shaker for 20min results in acceptable yields of 0.4 to 1.1mg. The overall process time for 96 sample is less than 1.5h. In order to reach its full potential the resin was incubated under saturating conditions with 4.7 mg target protein for 60 min. By increasing the shaking time during batch binding and protein load, as much as 2.9 mg of His-tagged protein per well could be obtained, with a purity above 90% (Figure 3). Even if one is looking for highest yields the overall process time is less than 2h for 96 samples. Since this effective purification system allows to use the maximum capacity of the resin the costs per sample are reduced. The elution from 96 identical samples is consistent from well to well. On average yield per well differs only by 3.4 % (relative standard deviation) indicating high well-to-well reproducibility (Figure 4).

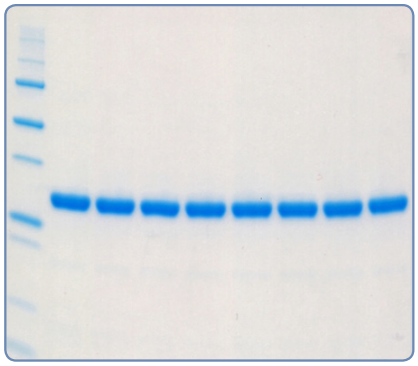

Figure 3: SDS-PAGE analysis

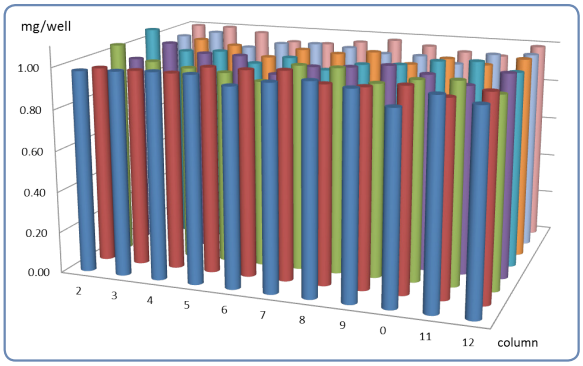

Figure 4: High reproducibility among different wells

Conclusion

MACHERY-NAGEL and Hamilton have co-developed a rapid His Tag protein purification method with highly reproducible and consistent results and good recovery rates. The yields per well only differs by ~4% (Figure 5). Cross contamination is reduced to a minimum because of an absolutely leak-free filter plate with a special designed filter frit material and long drips in combination with MACHEREY-NAGEL wash plate.

Loaded Sample [mg per well]	Yield [mg per well]	Batch Binding [min]	Time for 96 samples	Relative standard deviation
0,550	0,416	20	1 h 20 min	3,1%
0,700	0,530	20	1 h 20 min	3,1%
0,775	0,586	20	1 h 20 min	2,4%
1,600	1,144	20	1 h 20 min	3,7%
4,700	2,937	60	1 h 50 min	4,4%

Figure 5: Sample yields and Processing time

Configuration

ML STARlet	
width: 1200mm, height: 903mm, depth:795mm	
8 individual channel (1ml)	precision @ 10µl: 1.0% trueness @ 10µl: 1.5%
	precision @ 200µl: 075% trueness @ 200µl: 1.0%
Hamilton Heather Shaker	temperature range: up to 105°C
	shaking speed: up to 2500rpm
Hamilton CVS Vacuum System with MACHEREY-NAGEL insert frame	

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