Informational

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Protein A / Protein G Troubleshooting Guide

Agarose Beads

Introduction

Possible causes of problems that could appear during the purification protocol of immunoglobulins are listed below. The causes described are theoretical and it is always advisable to contact our team with your specific problem.

The table delineates the potential problems at each step in the protocol that might explain poor performance.

OBSERVATIONS	POSSIBLE CAUSES	RECOMMENDATIONS
TARGET PROTEIN NOT BOUND TO THE COLUMN	Conditions in binding or elution are not the optimum ones.	- Optimize pH, flow, temperature as well as salt or ion concentration
	Channels have formed in column bed so loaded sample runs through column without interacting with Protein A/Protein G	- Re-pack column.
	Column has not been stored in recommended conditions after previous usage	- Always follow manufacturer's recommendations.
	The antibody to be purified has a low affinity with Protein A/Protein G	-Look up bibliography on the subject and, if that observation is true, try an alternative way of purification.
	Protease presence	- Add protease inhibitors to sample loading / wash buffer. - Work at lower temperature (such as 4°C) to minimize degradation.
THE ANTIBODY IS DEGRADED	Antibody can be unstable in elution conditions	- Follow usage instructions neutralizing the fraction of the eluted antibody.
ANTIBODY IS NOT DETECTED IN THE ELUTION PROCESS	The IgG subclass doesn't bind to the resin	-Use another affinity column to purify the antibody

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BUBBLES IN THE PRE-PACKED COLUMN	Column poured and stored at one temperature, but used at another	-Equilibrate the column in the same temperature conditions as in the usage step.
	There are air bubbles in sample or buffer	-De-gas sample and all buffers used.
COLUMN FLOW IS VERY SLOW	There are air bubbles in sample or buffer that are blocking flow through pores.	-De-gas sample and all buffers used.