Table 2 Purification of recombinant METase from *Escherichia coli* JM109 harboring the plasmid pMGL-Trc03. (Adapted with permission from [46])

Purification step	Number of batch	Total activity (mU)	Total protein (g)	Specific activity (U/mg)	Yield (%)	Endotoxin (EU/mg)
Cultivation	2	83.3	3440	24	100	ND
Debris removal	2	81.2	2680	30	97	2×10^6
First crystallization	2	70.8	1320	54	85	6×10^4
Second crystallization	2	64.2	1170	55	77	1×10^4
Third crystallization	2	61.4	1100	56	74	8×10^3
DEAE-Sepharose FF	1	40.8	720	57	49	2
Sephacryl S-200 HR	3	34.1	600	57	41	8×10^{-1}

ND not determined, *DEAE* diethylaminoethanol, *FF* fast flow, *EU* endotoxin unit

small-scale experiments. Finally, the enzyme was purified with anion-exchange chromatography and polished with a gel filtration step to achieve sufficiently high-purity enzyme with significantly reduced endotoxin content for clinical applications (Table 2).

Crystalline Monoclonal Antibodies

Crystallization of mAbs or crystalline mAbs find applications particularly in three areas of structural biology, purification and process development, and high concentration or sustained-release mAb formulations.

Drug Discovery and Structural Biology Fab fragments and nanobodies have been used as chaperons in biology efforts to characterize difficult-to-crystallize targets such as membrane proteins. Bukowska et al. review new concepts and aids to facilitate protein crystallization, wherein the application of mAbs, mAb fragments, and camelid single-chain nanobodies as crystallization aids has been described [9].

mAb Crystallization: Purification Process Development Crystallization of proteins from clarified fermentation broth has been explored for enzymes such as lipase [27]. Such an approach has also been considered for complex biomolecules such as mAbs. Zang et al. describe the utilization of protein crystallization as a downstream processing step for a therapeutic antibody namely immunoglobulin G4 (IgG4) [50]. The purity of the crystalline mAb was found to be about 90%, though the crystallization yields were unsatisfactory. Bean and Matthews describe a similar crystallization-based approach for therapeutic proteins and antibodies for purification and