In recent times, nucleic acids self-assemblies have gained popularity as nanomaterials due to their ability to arrange themselves in a precise and resilient manner, offering specific shapes and versatile functions. Nonetheless, a significant obstacle remains in effectively purifying sizable amounts of DNA nanostructures or DNA-templated nanocomplexes for diverse applications. Current purification techniques often fall short in scalability or compatibility with tailored structures. To address this challenge, we present a dependable and scalable method for purifying DNA nanostructures utilizing Sepharose resin-based size exclusion. This approach enables manual column packing with the added advantage of reuse. Purification is accomplished through a gentle gravity flow process, wherein larger DNA nanostructures are initially eluted, followed by smaller impurities like single-stranded DNA and proteins. We have validated the efficacy of this method in purifying both DNA origami assemblies and protein-bound DNA nanostructures. In contrast to traditional agarose gel electrophoresis, which typically yields 1 μg or less of purified products, our technique can purify around 100 to 1000 μg of DNA nanostructures within 30 minutes, with consistent recovery rates ranging from 50% to 60%. The resulting purified nanocomplexes demonstrate improved precision in assessing enzyme functions and triggering antibody-mediated activation of complement protein reactions.