Accurate titer determination of recombinant proteins is crucial for evaluating protein production cell lines and processes. Even though enzyme-linked immunosorbent assay (ELISA) is the most widely used assay for determining protein titer, little is known about the accuracy of commercially available ELISA kits. We observed that estimations of recombinant human α1-antitrypsin (rα1AT) titer by Coomassie-stained SDS-PAGE gels did not correspond to previously obtained titers obtained by a commercially available ELISA kit. This prompted us to develop two independent quantification assays based on biolayer interferometry and reversed-phase high-performance liquid chromatography. We compared the rα1AT titer obtained by these assays with three different off-the-shelf ELISA kits and found that the ELISA kits led to inconsistent results. The data presented here show that recombinant protein titers determined by ELISA kits cannot be trusted per se. Consequently, any ELISA kit to be used for determining recombinant protein titer must be validated by a different, preferably orthogonal method.