urea, and autoclaving at 121°C for 15 minutes, several of which will not be suited if the target protein is present. Biological assays such as in vivo infection of susceptible animals are time consuming (months to years). They will not be of practical use in the test of biopharmaceutical products. The best semiquantitative biochemical assays include WB, capillary immunoelectrophoresis, conformation-dependent immunoassay, and dissociation-enhanced, time-resolved fluoroimmunoassay. The infectious dose is not known. Acceptable criteria must be decided upon on a case-by-case basis.

5.3.4.5 Proteolytic enzymes Proteolytic enzymes are released to the medium because of cell death, mechanical stress, or induced cell lysis. Their presence should be expected during fermentation and initial downstream unit operations. Measures are taken to work fast at low temperatures and to avoid working near the pH optimum of the enzyme. The most rewarding strategy is to prevent proteolysis already during fermentation either by use of mutant strains or by optimizing the conditions toward minimum enzymatic activity. Most enzymes of the vacuoles and lysosomes will be minimally active at slightly alkaline pH (7–9), a pH interval strongly recommended for extraction of proteins expressed in bacteria. Proteins are probably more resistant toward proteolytic attacks in their native state and stabilizing factors (e.g., cofactor, correct parameter interval, cosolvent) should always be considered optimized. The use of protein inhibitors is not recommended for safety reasons. Proteolytic enzymes are typically removed during the capture and intermediary purification steps and they are rarely copurified with the target protein throughout the downstream process. Despite the variety of enzymes present in the cell cytosol, proteolytic enzymes rarely constitute a problem in final products. Selective removal (e.g., affinity chromatography) of specific enzymes should be considered. Most proteins will be removed by means of 0.1–1 M NaOH (make sure that equipment, filters, and chromatographic media are not affected by NaOH). Suited analytical methods for early control are SDS-PAGE and western blotting. Purified preparations may be analyzed by means of HP-IEC, HP-RPC, MS, and peptide mapping. Ascertain that the degradation observed is not a function of the analytical assay. Enzyme inhibitors can be used for prevention of enzymatic activity in analytical assays.

5.3.4.6 Lipids Lipids (lipoproteins, triglycerides, phospholipids, cholesterol) are brought to the medium by cell lysis. If transgenic animals are used, the protein is expressed in the milk containing up to 4% fat. Lipids can be removed from the feedstock by centrifugation, by specific adsorption to hydrophobic compounds such as Hyflow by precipitation with dextran sulfate, by binding to anion exchangers, or by affinity chromatography allowing for specific binding of the target protein. Lipids will bind to hydrophobic media and surfaces. Lipids are retarded (two to three column volumes) by adsorption to Sephadex. Milk fat is usually removed by centrifugation. Lipids are removed by means of NaOH or