Assay: proteins with disulfide bridges can be fluorescently-tagged. Mixtures of protein that includes the protein of interest is mixed with fluorescently-tagged proteins that are high in disulfide bridges. If the level of fluorescence decreases, then the fluorescently-tagged proteins that are high in disulfide bridges are being degraded, indicating that the protein of interest -- a protein in the lysosome that degrades proteins with disulfide bridges -- is present.

1. Cell fractionation is performed by centrifuging with gradual increase in rotation speed, with the nuclear pellet being the first pellet to form initially. The supernatant is centrifuged under increased speed to isolate the mitochondrial pellet. The remaining supernatant is centrifuged, resulting in the microsomal pellet and the microsomal supernatant.
2. The microsomal supernatant and microsomal pellet are assayed. The pellet is expected to have more of the protein of interest, since the lysosome is a heavy organelle that is likely to be spun out of the supernatant into the microsomal pellet.
3. Lysine has a high pI, 9.74. The protein is also highly soluble. The microsomal pellet is put into an acidic solution. The pH of the solution is gradually raised, stopping in-between intervals to collect any protein that have precipitated out of solution. Any protein that precipitated at a pH under 9 are unlikely to be the protein of interest and are, therefore, discarded. The pH is then raised to 9.74. Since the protein is still highly soluble, the protein did not precipitate significantly out of solution. Therefore, most of the precipitates at this pH may be other proteins. These proteins get filtered out of solution.
4. Large amounts of salt is added to the solution to precipitate out the protein. The precipitates are filtered out of solution and then assayed.
5. The protein, being 56 kDa in size, is also small. Thus, the precipitates are dissolved in solution again and subjected to gel filtration column chromatography, with the later fractions being collected.
6. Part of the mixture is mixed with a protease, and the peptides are sequenced.
7. The remaining mixture is mixed with a second protease and then sequenced.