# FAQ

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## Enzyme Units FAQ

### For proteinase K and other proteases

### How does an enzyme unit describe activity?

An enzyme is usually valued for its function and activity rather than its mass, therefore enzyme units serve as a quantification of enzyme activity. For many enzymes, the activity under specified conditions can be expressed in *International Units* (IU), which is defined as the amount of enzyme that converts 1 µmol of a given substrate to a given product per minute.

### What is specific activity?

Specific activity is defined as the enzyme activity per mass of protein and is seen as a measure of an enzyme's purity.

### How are enzyme units defined for Proteinase K?

For enzymes that use large non-specific macromolecules as substrates (such as amylases or proteolytic enzymes), defining the molecular mass of the substrate can be tricky because in actuality the substrate changes each time a bond of the macromolecule is cleaved by the protease. Activity for these types of enzymes are usually determined by measuring the change in color intensity of the protein splits products per minute and using a standard curve to estimate the concentration of those products.

In 1938, M. L. Anson used denatured hemoglobin as a substrate for proteolytic enzymes in a Lowry assay. The undigested protein was then precipitated and the concentration of "protein split products" was estimated colorimetrically. Thus, the term Anson units was popularized and mAnson Unit was defined as the amount of enzyme that liberates 1 µmol of TCA-soluble, Folin-positive amino acids within 1 minute at pH 7.5 and 37°C, using hemoglobin as a substrate. In 1958, Hagihara decided to use casein as a substrate instead of hemoglobin. The term protease units is commonly used and can be defined in the same manner as mAnson units, except using casein as the substrate. mAnson units (that use hemoglobin as a substrate) are considered equivalent to protease units (that use casein as a substrate) when defining the activity of Proteinase K or other proteolytic enzymes.

### What is a Folin-positive amino acid?

After digestion of a protein with Proteinase K, the protein is precipitated with TCA. The amino acids (protein split products) that remain in the solution are incubated with Folin-Ciocalteu reagent (FCR). FCR primarily reacts with tyrosine, but also tryptophan and cysteine so each of these amino acids would be considered Folin-positive. A standard curve is generated using tyrosine and the concentration of TCA-soluble, Folin-positive amino acids is estimated by

comparing the color intensity of the protein split products with the color intensity of the tyrosine standards.

## References

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