



Enzyme units, activity and specific activity explained

There is much confusion over enzyme units, enzyme activity and specific activity. This guide seeks to explain these concepts in simple terms so that you will easily be able to form an opinion about the quality and value of competing product offerings.

The standard definition of the enzyme unit is given below:

*1 unit (U) is the amount of enzyme that catalyses the reaction of 1 **mmol** of substrate per minute (definition A)*

However, suppliers of enzymes seldom use this definition as it frequently requires labels to be written in fractions of units or converted into milli-units. The following definition is more commonly employed:

1 unit (U) is the amount of enzyme that catalyses the reaction of 1 nmol of substrate per minute (definition B)

While the definitions may vary the user can still make valid product comparisons as long as suppliers are explicit about their unit definitions and the assay conditions employed.

How do I compare enzymes from different suppliers?

There are several things to consider here: cost per unit, activity, specific activity and the assay conditions that the supplier has used.

Cost per unit:

Let us assume that a vial contains 1 unit of enzyme, using definition A. The same vial would be labelled 1000 units if definition B were employed. While the label is different, the contents of the vial clearly are not. If the cost per unit from one supplier appears to be 500-2000 fold cheaper than from another it is extremely likely that the unit definitions are different. To ensure that you are comparing like with like, check the unit definitions and express the number of units in each case as *either* nmol per min *or* μ mol per min. Remember to divide the nmol per min values by the cost per vial if the pack sizes are different.

If the calculated cost per unit varies more than 2-3 fold one of the products may represent better value (i.e. you will get more assays per £ or \$ spent), but this is not necessarily the case (see below).

Factors that influence activity

In the above section we discussed unit definitions, which can markedly affect the number of units quoted on otherwise identical vials from different suppliers. In this section we discuss other factors that may lead to more subtle differences in the quoted values.

The conditions under which different suppliers carry out their assays may lead to genuine differences in the reported activity values but in relation to the number of assays that you will be able to carry out in your own laboratory these differences may be real or illusory. For example, assays may be carried out between 20-37°C. While it is not unreasonable for a supplier to use a temperature of 37°C it should be noted that the same assay carried out at 20°C would give significantly fewer units. The definition of enzyme units is better expressed thus:

1 unit (U) is the amount of enzyme that catalyses the reaction of 1 nmol of substrate per minute under standard conditions.

Unfortunately, the meaning of 'standard conditions' is somewhat vague. The assay temperature and the concentrations of substrates, buffers, cofactors, and other additives may all impact the measured number of enzyme units. For example, if the K_M for the substrate is 0.1 mM, assays carried out at 1 mM substrate will give almost twice the number of units as assays carried out at the K_M concentration, even though the amount of protein added to the assay is the same.

It follows therefore that vials of enzyme that are identical (in terms of quoted units) may not necessarily give the same number of assays. Conversely, a vial of 100 units of an enzyme measured by the supplier at relatively low temperature and low substrate concentration may actually give more assays under your preferred conditions than a vial quoted as 150 units, but measured by the supplier at 37°C with excess substrate.

It is unlikely that the assay conditions used by the supplier will be identical to your own preferred conditions. However, you should be aware that if the supplier has run assays at 37°C and you plan to run assays at 20-25°C, as is the case in many drug screening labs, it would not be surprising if you obtained fewer assays than expected. You might of course increase the assay time to compensate for the lower activity under your conditions.

In summary, some of the differences in quoted units may be illusory and others will genuinely reflect the different assay conditions used. All reputable suppliers will state, at an absolute minimum, the definition of the enzyme unit, the temperature at which the assay was performed, and the full composition of the assay buffer. Without this information you will not be able to replicate the supplier's assay or extrapolate to your own assay conditions.

What is activity and how do you determine the amount of enzyme needed for an assay?

Activity is quoted as units per ml, in other words nmol per min per ml (assuming that unit definition B has been adopted). Two vials of enzyme can contain the same number of units in total but have different activities.

In practice, it is extremely rare for users to calculate the number of units required for an assay either because it is difficult to replicate the assay conditions used by the supplier or because different assay conditions are required in the user's lab. Moreover, some activity may have been lost in storage or during transportation so it is sensible to confirm the actual number of units per ml in your own lab prior to setting up large-scale experiments. This is a simple task: a small amount of enzyme is serially diluted (e.g. log dilutions) and a fixed volume of each dilution is assayed for enzyme activity. A second set of dilutions can then be prepared in order to home in on a suitable dilution.

What is specific activity?

Specific activity is the number of enzyme units per ml divided by the concentration of protein in mg/ml. Specific activity values are therefore quoted as units/mg.

Specific activity is of no relevance as far as setting up assays is concerned, though it is an important measure of enzyme purity and quality (see below). Activity values (units/ml) are far more important for assay set up since the amount of substrate converted is determined by the number of enzyme units added. Note: It is impossible to calculate the volume of enzyme required for an assay from the specific activity value alone, since the specific activity values for an undiluted enzyme and a 1/1000 dilution are *identical*. Both the units per ml and mg per ml have been reduced by the same factor.

Quality control

There are two key parameters when considering quality of enzymes. Specific activity values are important because different batches of a pure enzyme should always exhibit, within experimental error, the same specific activity value. (Note: as specific activity is dependent on enzyme unit definitions some care is required when comparing specific activity values from different suppliers). Batches that are below the expected specific activity value may contain impurities or may be electrophoretically homogeneous but with a population of molecules that have become denatured. A second key parameter is purity by SDS-PAGE. Equipment for running gels is ubiquitous thus if the supplier of an enzyme does not show a gel for each batch then you might like to ask why not!

Innova sells a range of enzymes and assay kits for drug discovery and basic research. Check out our web site using the link below.



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