

Items to be included in the test report for validation of the enzyme activity determination method

1 Title (including the name of the target substance and test items, Example: Validation report of the enzyme activity determination method for xxx)

2 Date of report

3 Author

4 Testing institution (e.g., institution name, address, phone number, email address)

5 Purpose (Example: To validate that the method we established can yield stable test results for determination of the enzyme activity of xxx by showing the enzyme reaction conditions and the details that have been examined by the quantitative method of the reaction products.)

6 Determination method

(1) Outline of the determination method

Describe the principle of the enzyme activity determination method and cite and attach any references.

Examples of a reference and items included in it:

- A reference on enzyme activity determination methods, with author name, article title, journal name, year of publication, volume (issue), and page numbers
- A reference on the quantitative method of the products of enzymatic reaction: author name, article title, journal name, year of publication, volume (issue), and page numbers

(2) Details of the determination method

(a) Reagent chemicals and reagent solutions

- Grade of the substrate and other reagent chemicals used in the test (e.g., catalog numbers)
- Grade of the standard enzyme used as a positive control (e.g., catalog number)

(b) Equipment

(c) Method of handling of test items (Describe in detail the items to be included in the draft specifications.)

- How to prepare the sample solution and substrate solution
- How to handle enzyme reactions
- Quantitative method of reaction products

(d) Calculation of enzyme activity (formula) and its explanation

Include the definition of enzyme units and enzyme activity specification values (proposed).

(e) Flowchart of the determination method (strongly desirable)

(3) Rationale for establishing the determination method

Describe in detail the rationale used to establish the determination method.

If you performed some experiments, indicate their contents and results and describe the reasons for your determination; include the raw data so that we can see the process.

If you want to cite a reference, add an excerpt of the content.

4_ Items to be included in the test report for validation of the enzyme activity determination method

- (a) Details of preparation of sample solutions (e.g., diluent, dilution factors)
- (b) Details of enzyme reaction conditions (e.g., substrate concentration, reaction conditions such as pH, temperature, time)
- (c) Details of the quantitative method of reaction products
 - Method of handling
 - Calibration curve (e.g., concentration range of linearity, correlation coefficients)
 - If HPLC or GC is used, chromatograms of the reference standard and the test sample should also be submitted.
- (4) Other items considered

7 Validation of the determination method

To validate the enzyme activity determination method, perform the following tests.

Make sure you describe the sample used in the test to show the sample's origin (such as the name, manufacturer, date of production, and lot number).

- (a) Prepare sample solutions with different dilution factors from a single sample (setting concentrations at the center of the calibration curve and at points close to the lowest and highest concentrations; perform five trials per sample), measure the enzyme activity, and show the results (per sample) by using raw data (e.g., absorbance).
- (b) Determine and compare the average value and relative standard deviation of the enzyme activity at each dilution factor.

8 Considerations

Describe your thoughts regarding the performance of the enzyme activity determination method.