{***Attention to readers: This Check-sheet 2 is prepared by translation into English from a non-official edition of Check-sheet 2 in Japanese（ver. 20240920）***}

Specific considerations for completing Check-sheet 2

1) To create the Overview document, confirm whether the information is appropriate for use for your claims. Also confirm the rationale behind your outline of the target substance, your key specification points, your outline of the standards for use and safety, etc.

2) In III (Findings regarding safety), descriptions should be based on the Guideline for the Risk Assessment of Additives (Enzymes) (2021) issued by the Food Safety Commission of Japan (FSCJ) (hereinafter referred to as "the FSCJ Guideline (Enzymes)").
(https://www.nihs.go.jp/dfa/FADCC/dfa_e_fadccsite/e_img/e_guidelines_Enzymes.pdf)

3) Before you start drafting the Overview document, you should check the completeness of the references, considering the framework and narrative of your Overview document. For this procedure, you need to collect the references and then write down the keypoints of each reference in this Check-sheet 2 to confirm the appropriateness of your preparation. If you prepare the Overview document before you have all the references, you may be faced with a lack of references to base your description on later. Please submit the references on which the content of the Overview document is based along with the Overview document at the time of application.

4) At the beginning of this Check-sheet, enter the name of the target substance. As Check-sheet 2 may be submitted multiple times, use an expression such as "First Draft" for the first version submitted, "Second Draft" for the second, etc.

Detailed information on the documents submitted

1) By referring to the Procedure for Preparing Application Documents for Designation of Food Additives and Revision of Use Standards for Food Additives (hereinafter, the Procedure) and Handbook for the Procedure, provide the number of references that will serve as the basis for each item on the Check-sheet, as well as an outline (relevant content) of each reference.

2) The references on this Check-sheet may include not only printed matter, such as research reports published in academic journals and publications, but also the results of searches of the relevant laws, regulations, and database information on the Web. When selecting references, keep in mind the fact that the FADCC usually considers the reliability of the source according to the order below, with (A) as the most reliable. Please try to obtain original papers whenever possible.

   A) Public information such as reports by specialized public institutions (including on their websites)
   B) Reviewed academic papers in specialized fields
   C) Books in specialized fields
   D) Test data provided by business operators and analytical institutions
   E) Articles published in newspapers and magazines

F) Web articles other than A) to E)

3) As a great deal of relevant references on which the items on the Check-sheet may be based may be found, number the references serially and list them in V (Information on references). Enter a reference number corresponding to each reference (You may also write the title of the reference next to the reference number.) Briefly describe in the Outline the relevant content of each part. Such information will help you to create the draft Overview document and list the references.

4) If there are a number of references for a given Check-sheet item, don't restrict yourself to just one: augment the Reference numbers and the Outline by adding lines as needed.

5) In the Outlines in I-1-1 (Name) of I (Outline of the food additive), enter both Japanese and English names. If you are applying for designation of a new additive, use the Japanese and English names (such as the names of the principal ingredient in the food additive) that you wish to use for the food additive.

6) In the Outline in I-2 (Origin or details of development), provide concise and relevant information by using your references. For example, "XX was isolated from wine in Germany by Weber et al. in 1890, and industrial production was later initiated by Newton et al."

7) For I-3 (Conditions of use in Japan and overseas), list the relevant laws and regulations used to confirm the approval or registration of application substance (proposed food additive) as references in the order of the international organizations/national agencies listed in the examples. If you cannot get any information on application substance, you may provide approval/registration information of related compounds as food additives in (Country/Region) under (Law Name) as references. As laws and regulations are subject to revision, make sure you provide the latest information. If such information is not found, convert your search records, including the search engine name, search term, search date and search results (search screen), into pdf format and list them as references. In the Outlines, describe a description of your search method and the results of your search. If you would add the information on the situation on the registration/designation of application substances in the country/region other than designated 5 countries/regions, please list up them after the item I-3-5 (Australia and New Zealand) as same manner.

8) For I-4 (Safety evaluations by international organizations), as reevaluations of additives may be conducted by these organizations, make sure you provide the latest information. If you mention a country or region in I-3 (Conditions of use status in Japan and overseas) other than the ones listed in sections I-3-1 (Japan) to I-3-5 (Australia and New Zealand), list the respective assessments as references.

9) In I-4-1 (FSCJ, Food Safety Commission of Japan), additives may be evaluated not only as food additives but also as pesticides and feeds, fertilizers, etc. The evaluation reports may be referenced on the FSCJ's website. Please try to collect this information. Before the FSCJ was established in July 2003, such evaluations were conducted by the Pharmaceutical Affairs and Food Sanitation Council of the Ministry of Health, Labour and Welfare (or its predecessor, the Food Sanitation Council of the Ministry of Health and Welfare).

10) In preparing I-5-4-1 (Draft specifications), collect the relevant latest information on specifications from the Combined Compendium of Food Additive Specifications (JECFA), FCC (Food Chemical Codex), EU, JP (Japanese Pharmacopoeia), Japan's Specifications and Standards for Food Additives (hereinafter referred to as JSFA), etc., as well as that for similar substances. Use the relevant latest information on specifications. If you need to translate them in Japanese, make sure you do it accurately, and prepare a comparison table of draft and existing specifications. (The comparison table should be included in the Overview document but does not have to be included in Check-sheet 2). Define the specifications used for the draft specifications as "Reference specifications," and enter the reference in I-5-4-1-1 (Reference specifications). If there are multiple Reference specifications, you may augment the Reference numbers and the Outline by adding lines as needed.

11) Tick the relevant items in the check boxes of I-5-4-1-2 (Information on which the draft specifications are based (preparation checklist)) in order to monitor the progress of establishment of specifications. For (i) Purity, please increase the number of rows according to the number of items to be set and provide the name of each item.

12) In Sections (h) Identification and (k) Assay of enzymatic activity I-5-4-1-2, "Developed in-house" refers to the establishment of a newly developed in-house test method or to test methods partly modified from those established in JECFA specifications, FCC specifications, Japanese Pharmacopoeia (JP), JSFA, etc. "Existing method" refers to the use of an existing method (such as those of the JECFA, FCC, and JP) already established for similar food additives. In the case of Developed in-house, if a test method established for other additives is used as a reference, the referenced specifications (JECFA specifications, FCC specifications, JP, JSFA, etc.) should be used as the "Ref. Sp.". In "Validation of the method" under (l) Identification and (r) Assay, data, etc., are required to show that the method has been correctly confirmed or quantified.

13) For items other than (h) Identification and (k) Assay of enzymatic activity in Section I-5-3-1-2, validity must be confirmed when questions arise regarding the reliability of the test methods. Accordingly, it is desirable that the test method to be established has been validated at Stage 2. If the selected method is found to be inappropriate at Stage 4, the procedure will return to Stage 3.

14) In I-5-4-2-1 (Information on referred specifications), among the references collected in 11), the ones included in the comparison table are listed. Please provide additional Reference numbers and Outline lines as appropriate.

15) In I-5-4-3 (Rationale for establishing draft specifications), if multiple references (e.g., standard values, test methods, etc.) are referenced for the draft specifications, please provide additional Reference numbers and Outline lines as appropriate.

16) In I-5-4-4 (Verification data of draft test methods and test results), some test methods need to be verified. For example, when standard values are established in a purity test, the recovery rate and relative standard deviation in recovery testing (e.g., n = 3 to 5) must be reported. Organize and provide data (calibration curve, quantitative lower-limit value, etc.) that show the details of the testing procedure and data to establish the proposed specifications and testing methods. Create a summary report and cite these data in the summary report.

For all items, test results (usually 3 lots, 3 trials each) using the established test method are required. Please document the details of the implementation method and other data showing the process of obtaining the results in a report. The reports should then be included in references.

For Verification data of draft test methods and test results, please refer to the Chapter 5. (3)4) (試験法案の検証データ及び試験成績) in the Handbook for the Procedure (I-2.添加物の概要（2） ※Japanese only) published by FADCC and pdf file of (Items to be included in the test report) on the FADCC website.

17) In I-5-5 (Stability of the food additive), list the relevant references regarding the preservation and management of the target food additive. In II-2 (Stability of the additive in foods) of II (Findings regarding effectiveness), list the relevant references regarding the stability of the target food additive when it is added to food.

18) In I-5-6 (Method of analyzing food additives in food), include the reference used to set the analytical methods, the validation report of the analytical methods, and the results of analysis of the food additives in foods. If you have any references on the analytical methods for analyzing the additives in foods, you may include them in your references. However, developing an analytical method for the enzyme in foods might be difficult. In such a case, please provide a reasonable explanation of the difficulty and/or unnecessity of setting up the analysis for the enzyme, in the Outline in I-5-6-1 (Referenced papers, notification analysis methods, etc).

19) In I-6 (Draft standards for use), regardless of whether you wish to propose standards for use or don't wish to do so (as you consider such standards unnecessary), please list the relevant references.

20) Prepare for, and complete, each item in III (Findings regarding safety) on the basis of the FSCJ Guideline (Enzymes)) If relevant references in III were not found after a search and investigation, briefly describe what kind of investigation or search was performed and include this description as references.

21) Your claims about the results of toxicological testing in III must be based on the references. List any relevant references in a reference list.

22) The items in IV (Estimation and consideration of the daily intake) should be described in accordance with the FSCJ Guideline (Enzymes)). Collect and list references you wish to use to make claims based on standards for the proposed use (including rational grounds for estimation when proposed standards for use are not set) and on the dietary intake of Japanese, etc.

23) In V (Information on references), please include the reference numbers and bibliographic information of all documents listed as references in I (Outline of the food additive) to IV (Estimation and consideration of daily intake). When describing bibliographic information, please refer to the Chapter 4-1 (Regarding cited references) in the Handbook for the Procedure General notes published by FADCC.

24) If you have questions regarding preparation of the Overview document, refer to the Procedure, the FSCJ Guideline of Enzyme, the Consumer Affairs Agency website, and the FADCC's website, or contact us via email (address on the website).

About submission

When submitting Check-sheet 2, send the 6th and subsequent pages of Check-sheet 2 in digital format (MS Word is preferred) to the FADCC. Pages 1 to 5 contain points to note when filling out the document; you don't need to submit them.

Check-sheet 2

Food additives in general

New designation or revision of standards for use or specifications

Name of target substance:

Version:

Date of entry (year/month/day):

Applicant information

If the information is the same as on Check-sheet 1, enter only the name and affiliation of the contact person, then skip to I (Outline of the food additive).

Name of applicant or agent (company or organization name, etc.):

Address of applicant or agent:

Name and affiliation of contact person:

Phone number:

Email:

I. Outline of the food additive

I-1. Name and purpose of uses

I-1-1. Name

Reference number:

Outline:

I-1-2. Enzyme Commission number, CAS registry number, etc.

Reference number:

Outline:

I-1-3. Purpose of uses

Reference number:

Outline:

I-1-4. How to use

Reference number:

Outline:

I-2. Origin or details of development

Reference number:

Outline:

I-3. Conditions of use in Japan and overseas

I-3-1. Japan

Reference number:

Outline:

I-3-2. CODEX

Reference number:

Outline:

I-3-3. EU

Reference number:

Outline:

I-3-4. USA

Reference number:

Outline:

I-3-5. Australia and New Zealand

Reference number:

Outline:

I-4. Safety evaluations by international organizations

I-4-1. FSCJ (Food Safety Commission of Japan)

Reference number:

Outline:

I-4-2. JECFA (Joint FAO/WHO Expert Committee on Food Additives)

Reference number:

Outline:

I-4-3. EFSA (European Food Safety Authority) and SCF (Scientific Committee on Food)

Reference number:

Outline:

I-4-4. US FDA (Food and Drug Administration)

Reference number:

Outline:

I-4-5. FSANZ (Food Standards Australia New Zealand)

Reference number:

Outline:

I-5. Physicochemical properties

I-5-1. Source organism

Reference number:

Outline:

I-5-2. Methods of manufacturing

Reference number:

Outline:

I-5-3. Information on constituent (including reaction mode, mass, isoelectric point, amino acid sequence, temperature dependence, and pH dependence)

Reference number:

Outline:

I-5-4. Specifications

Note: If you require only the revision of standards for use, please go to I-5-4 (Stability of the food additive).

I-5-4-1. Draft specifications

I-5-4-1-1. Reference specifications

Reference number:

Outline:

I-5-4-1-2. Information on which the draft specifications are based (preparation checklist)

Check the box on the left for the item you want to set.

(For (a) to (s), refer to the Draft specifications in the Procedure)

Note: “Ref. Sp.” means “Reference specification”

Setting item	Draft specifications	Information	Check box	
☐	(a) Name (in Japanese)	Draft	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
☐	(b) English name	Draft	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
☐	Alternative English name	Draft	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
☐	(c) Alternative Japanese name	Draft	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
☐	(d) CAS registry number	Draft	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
☐	(e) Definition	Draft	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
☐	(f) Enzymatic activity	Draft (Specifications)	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
		Test results	☐ Established	☐ Not yet
☐	(g) Description	Draft (Specifications)	☐ Obtained	☐ Not yet

		Ref. Sp.	☐ Established	☐ Not yet
		Test results	☐ Obtained	☐ Not yet
☐	(h) Identification (Add rows if necessary.)			
	Items (Carbonates, potassium salts)	☐ Developed in-house ☐ Existing method		
		Draft (Test method and decision criteria)	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
		Validation of the method (when Developed in-house)	☐ Validated	☐ Not yet
		Test results	☐ Obtained	☐ Not yet
☐	(i) Purity (name of item(s) to be established; add rows if necessary)			
	Items (Lead, Arsenic, Residual solvent, etc.)	Draft (Standards and test methods)	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
		Test results	☐ Obtained	☐ Not yet
☐	(j) Microbial limits	Draft (Standards and test method)	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
		Test results	☐ Obtained	☐ Not yet
☐	(k) Assay of enzymatic activity			
		☐ Developed in-house ☐ Existing method		
		Draft (Method)	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet
		Validation of the method (Existing method is determined on an individual basis. For Existing methods, necessity is determined on a case-by-case basis.)	☐ Validated	☐ Not yet
☐	(l) Storage standards	Draft	☐ Established	☐ Not yet
		Ref. Sp.	☐ Obtained	☐ Not yet

I-5-4-2. Comparison table of draft and existing specifications

The purpose of the "Comparison table" is to confirm the proposed specifications and Reference Standards.

I-5-4-2-1. Information on referred specifications

Reference number:

Outline:

I-5-4-3. Rationale for establishing draft specifications

(a) Japanese name

Reference number:

Outline:

(b) English name and alternative English name

Reference number:

Outline:

(c) Alternative Japanese names

Reference number:

Outline:

(d) CAS registry number

Reference number:

Outline:

(e) Definition

Reference number:

Outline:

(f) Enzymatic activity

Reference number:

Outline:

(g) Description

Reference number:

Outline:

(h) Identification

Reference number:

Outline:

(i) Purity

Reference number:

Outline:

(j) Microbial limits

Reference number:

Outline:

(k) Assay of enzymatic activity

Reference number:

Outline:

(l) Storage standards

Reference number:

Outline:

I-5-4-4. Verification data of draft test methods and test results

I-5-4-4-1. Rationale for validity of draft test method(s) (For Validation data, usually 3 to 5 trials each)

Reference number:

Outlines:

I-5-4-4-2. Test results (usually 3 lots, 3 trials each)

Reference number:

Outline:

I-5-5. Stability of the food additive (results regarding storage management of the additive)

Reference number:

Outline:

I-5-6. Method of analyzing food additives in food

I-5-6-1. Referenced papers, notification analysis methods, etc.

Reference number:

Outline:

I-5-6-2. Validation of the analytical method(s) (e.g., recovery test results)

Reference number:

Outline:

I-5-6-3. Test results assayed for the proposed target food product (to the extent possible)

Reference number:

Outline:

I-5-6-4. Other reference(s) pertaining to analytical methods (not limited to the main analytical methods described in this document, but anything relevant that can be referenced)

Reference number:

Outline:

I-6. Draft standards for use

I-6-1. Draft standards for use

Reference number:

Outline:

I-6-2. Rationale for establishing draft standards for use

Reference number:

Outline:

II. Findings regarding effectiveness

II-1. Effectiveness as an enzyme, and comparisons of effects with those of other enzyme in the same category

Reference number:

Outline:

II-2. Stability of the Enzyme in foods

Reference number:

Outline:

II-3. Effects of the food additive on main nutrients in foods

Reference number:

Outline:

III. Findings regarding safety

III-1. Safety of the source organism

III-1-1. Pathogenicity and potential production of harmful substances

III-1-1-1. Pathogenicity of source organism

Reference number:

Outline:

III-1-1-2. Production of harmful substances by the source organism

Reference number:

Outline:

III-1-2. Parasitism and adhesion to humans or other organisms by the source organism

Reference number:

Outline:

III-1-3. Contamination of the source organism with pathogenic exogenous factors (viruses etc.)

Reference number:

Outline:

III-2. Degradability of the enzyme in the gastrointestinal tract

III-2-1. Data on degradability of the enzyme in the gastrointestinal tract

(Describe the following. The enzyme is easily broken down in the gastrointestinal tract. In principle, the enzyme's degradability should be confirmed to be degraded to a mass or less below which there is no longer any concern about allergenicity, using III-4.)

Reference number:

Outline:

III-2-2. Information on the main factors related to degradation in the gastro-intestinal tract

Reference number:

Outline:

III-2-3. Absorption of the enzyme or its degradation products, and the effect on absorption of the other nutrients

(Describe the following. When the enzyme is used in normal conditions and in an appropriate amount, the enzyme and its degradation products are absorbed into the body at a similar level to that of the food materials, and the

absorption of other nutrients is not inhibited.)

Reference number:

Outline:

III-2-4. Problem involving overdose of major components of enzyme

(Describe the following. When food utilizing the enzyme is ingested, the enzyme and its degradation products do not cause an overdose of the main enzyme components.)

Reference number:

Outline:

III-2-5. Excretion and accumulation of undegraded or partially degraded enzymes

(Describe the following. When food utilized the enzyme is ingested, the undegraded or partially degraded enzymes in the gastrointestinal tract are not excreted in the feces in large amounts, and undegraded or partially degraded enzymes do not accumulate in the body.)

Reference number:

Outline:

III-3. Enzyme toxicity

III-3-1. Repeated dose toxicity studies (90 days)

Reference number:

Outline:

III-3-2. Genotoxicity study

Reference number:

Outline:

III-3-3. Allergenicity

III-3-3-1. Four considerable points

III-3-3-1-1. Allergenicity of the source organism

Reference number:

Outline:

III-3-3-1-2. Allergenicity of the enzyme

Reference number:

Outline:

III-3-3-1-3. Changes in the physicochemical properties of the enzyme

Reference number:

Outline:

III-3-3-1-4. Structural homology of the enzyme with known allergens (that is, proteins showing allergenicity, including its relation, if any, to gluten induced enteritis)

Reference number:

Outline:

III-3-3-2. Examination of the IgE binding activity of the enzyme when allergenicity concerns cannot be denied from III-3-3-1

III-3-3-2-1. Examination results of a study using serum with a high specific IgE antibody titer against the organism in cases where the source organism is allergenic.

Reference number:

Outline:

III-3-3-2-2. Examination results of a study using serum with a high specific IgE antibody titer against the organism containing the allergen in case the structural homology with a known allergen is observed for the enzyme.

Reference number:

Outline:

III-3-3-2-3. Examination results of a study using serum with a high specific IgE antibody titer against a related species of the base organism in case the appropriate serum could not be obtained for the cases of III-3-3-2-1 and III-3-3-2-2.

Reference number:

Outline:

III-3-3-2-4. Examination results of a study using serum with a high specific IgE antibody titer against major allergens (egg, milk, soy bean, rice, wheat, soba(buck-wheat), shrimp, crab and/or peanuts) in case the appropriate serum could not be obtained for the cases of III-3-3-2-1, III-3-3-2-2 and III-3-3-2-3.

Reference number:

Outline:

III-3-3-3. When potential concerns of allergenicity cannot be excluded, even given the testing described in III-3-3-1 and III-3-3-2, potential allergenicity must be comprehensively evaluated, taking into consideration the data from clinical studies such as skin and oral tolerance testing.

Reference number:

Outline:

III-4. Examination of the enzyme for the degradability in the gastrointestinal tract and the allergenicity

It shall be clarified whether the following treatments III-4-1. to III-4-3. affect the characteristics of the enzyme, such as molecular weight, enzyme activity, and immunoreactivity.

III-4-1. Acid treatment and enzymatic (pepsin) treatment in simulated gastric juice

Reference number:

Outline:

III-4-2. Alkaline treatment and enzymatic (pancreatin) treatment in simulated intestinal juice

Reference number:

Outline:

III-4-3. Heat treatment (under the same or similar heating condition used in the treatment for human oral intake)

Reference number:

Outline:

IV. Estimation and consideration of the daily intake

Reference number:

Outline:

V. Information on references

1)

2)

3)

...

VI. Information on the testing institution(s)

Provide contact information to enable the FADCC to inquire about test results pertaining to the application. If the tests were conducted at different testing institutions, provide contact information for the person or department in charge at each institution.

Example:

Testing institution that conducted tests using in-house standards

Name:

Address:

Name and affiliation of contact person:

Phone number:

Email:

Testing institution that validated the analytical method on the basis of the draft standard testing method

Name:

Address:

Name and affiliation of contact person:

Phone number:

Email:

Testing institution that validated the method of analyzing food additives in food

Name:

Address:

Name and affiliation of contact person:

Phone number:

Email:

Institution that conducted the safety study or studies

Name:

Address:

Name and affiliation of contact person:

Phone number:

Email: