



## Enzyme REACH Consortium Guidance: How to populate IUCLID Section 1 for enzymes

### Disclaimer

This communication is meant as guidance only. It is published by Enzyme REACH Consortium (ERC) in order to assist its members in their efforts to understand and comply with REACH.

Please be reminded, however, that REACH is the only authoritative legal text and that the present document does not substitute legal or otherwise expert advice. ERC and its members do not accept any liability for use of this communication or for activities contemplated and carried out under or relying on this communication.

### Introduction

The purpose of the guidance is to give practical information to Enzyme REACH Consortium (ERC) members based on our experiences with REACH dossiers. The guidance focuses on specific descriptions on enzyme substances which may not be covered by ECHA's support documents.

ECHA has specifically addressed the issue of identification of enzyme substances, cf. section 4.3.2.3 of ECHA's Guidance on Substance Identification<sup>1</sup>. IUCLID should be populated according to the identification. The relevant paragraphs of this guidance are inserted below. This guidance does not cover issues related to split and merge SIEFs.

*The enzyme substance should be regarded as a 'UVCB1-substance' due to its variability and partly unknown composition.*

...

*Enzyme substances are identified by the enzyme protein (IUBMB nomenclature) and the other constituents from the fermentation.*

...

*The enzyme substance typically contains 10-80 % (w/w) of the enzyme protein. The other constituents vary in percentage and depend on the production organism used, the fermentation medium, and operational parameters of the fermentation process as well as the downstream purification applied, but the composition will typically be within the ranges indicated in the following table.*

<i>Active enzyme protein</i>	<i>10 - 80%</i>
<i>Other proteins + peptides and amino acids</i>	<i>5 - 55%</i>
<i>Carbohydrates</i>	<i>3 - 40%</i>
<i>Lipids</i>	<i>0 - 5%</i>
<i>Inorganic salts</i>	<i>1 - 45%</i>
<i>Total</i>	<i>100 %</i>

<sup>1</sup> Guidance for identification and naming of substances under REACH and CLP. Reference: ECHA-16-B-37.1-EN. Version 2.1, May 2017



## How to read the guidance

The guidance text is black.

Proposed text populated in IUCLID is blue

Text which is variable depending on enzyme is orange.

## Section 1. General Information

### Section 1.2 Substance Composition

Several ERC policy documents argue that enzyme sameness is only dependent on the enzyme protein, not the constituents. Nevertheless, this section should be populated in order to pass the Technical Compliance Check. ERC recommends populating this section according to ECHA's guidance on substance identification Section 4.3.2.3 (page 1 of this guidance).

Constituent (Reference substance should be created accordingly)	Range	Typical concentration
Active enzyme protein of the enzyme	10 - 80%	50 %
Other proteins + peptides and amino acids	5 - 55%	30%
Carbohydrates	3 - 40%	10 %
Lipids	0 - 5%	1 %
Inorganic salts	1 - 45%	9 %
Total	100 %	100 %

Please see Remarks for each component below.

#### Active enzyme protein of the enzyme

Remarks: In accordance with "GUIDANCE FOR SUBSTANCE IDENTIFICATION AND NAMING IN REACH Section 4.3.2.3", the enzyme substance consists of 1) the active enzyme protein and 2) constituents other than the active enzyme protein.

1) Enzyme substance: The enzyme substance is identified according to catalytic activity defined by IUBMB (INTERNATIONAL UNION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY (<http://www.chem.qmul.ac.uk/iubmb/>)).

IUBMB name: alpha-amylase; Enzyme Class No.: 3.2.1.1; Reaction: Endohydrolysis of (1→4)-α-D-glucosidic linkages in polysaccharides containing three or more (1→4)-α-linked D-glucose units

2) Constituents other than enzyme protein. The substance does not contain a constituent which is ≥ 10 % (w/w) or relevant for classification and labelling and-or PBT assessment. The enzyme



substance typically contains 10-80 % (w/w) of the enzyme protein. The other constituents vary in percentage and depend on the production organism used, the fermentation medium, and operational parameters of the fermentation process as well as the downstream purification applied, but the composition will typically be within the following ranges: Active enzyme protein 10 - 80%, Other proteins plus peptides and amino acids 5 - 55%, Carbohydrates 3 - 40%, Lipids 0 - 5%, Inorganic salts 1 - 45%. The enzyme substance is produced by organisms which meet the criteria for "Safe Strain Lineage Concept" in "Safety evaluation of technical enzyme products with regards to the REACH legislation" dated September, 2021, published by Enzyme REACH Consortium (<http://www.enzymes-reach.org/>). The constituents other than enzyme protein produced by the organisms meeting the above criteria do not contribute to classification, thereby the enzyme substance having the same catalytic activity from such safe organisms are considered as the same substance.

### Other proteins + peptides and amino acids

Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various proteins and peptides.

### Carbohydrates

Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various carbohydrates.


### Lipids

Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various lipids.

### Inorganic salts

























Description: It is a chemical group as a constituent of enzyme substance derived from the fermentation or extraction process (section 4.3.2.3 Guidance for Substance Identification and Naming in REACH). It consists of various inorganic salts.

**Degree of purity**



ca.   % (w/w)

**Constituents**

				
ca. 50 % (w/w) alpha-amylase (active enzyme protein) / Active enzyme protein of alpha-amylase (EC no. 232-565...				
ca. 30 % (w/w) Protein as a constituent of enzyme deriving from the fermentation or extraction process / Protein ...				
ca. 10 % (w/w) Carbohydrates constituent of enzyme deriving from the fermentation or extraction process / Carb...				
ca. 1 % (w/w) Lipids as a constituent of enzyme deriving from the fermentation or extraction process / Lipids as a...				
ca. 9 % (w/w) Inorganic salts as a constituent of enzyme deriving from the fermentation or extraction process / In...				



## Section 1.4 Analytical Information

ERC recommend that assay method(s) for each constituent is populated. Spectra data are not relevant for active enzyme protein and other constituents.

Constituent (Reference substance should be created accordingly)	Analytical method
Active enzyme protein of the enzyme	Enzyme activity assay
Other proteins plus peptides and amino acids	Any method e.g. Kjeldahl to determine protein, peptides and amino acid
Carbohydrates	Any method e.g. colorimetric assay to determine carbohydrates or calculation
Lipids	Any method to determine lipids
Inorganic salts	Determination of ash

In each assay, a method should be uploaded and results should be populated.

### Analytical information

Analytical methods and spectral data

Optical activity

### Results of analysis




	<span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: green;">+</span>
Enzyme activity assay	<span style="color: blue;">↕</span> <span style="color: grey;">↕</span> <span style="color: blue;">↕</span> <span style="color: green;">+</span>   <span style="color: red;">✖</span>
Protein with relevant assay method	<span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: green;">+</span>   <span style="color: red;">✖</span>
Carbohydrate with relevant assay method	<span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: green;">+</span>   <span style="color: red;">✖</span>
Ash	<span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: green;">+</span>   <span style="color: red;">✖</span>
Lipid	<span style="color: blue;">↕</span> <span style="color: blue;">↕</span> <span style="color: grey;">↕</span> <span style="color: green;">+</span>   <span style="color: red;">✖</span>



## Enzymes REACH Consortium

enzymes-reach.org

Protein with relevant assay method ⬆ ⬇ ⬇ ⬆ | ✖

Analysis type	Protein with relevant assay method
Tested substance	Protein as constituent of enzyme deriving from the fermentation or extraction process
Method used	Determination of Protein
	 Analytic method description_Protein_PSL-SM-1010.01-D.doc.pdf / 137.11 KB  
Remarks	result : 14400 mg/L 