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Product Information

Pepsin-Agarose, from porcine gastric mucosa
Lyophilized powder

Catalog Number **P0609**
Storage Temperature –20 °C

Product Description

Pepsin (EC 3.4.23.1) is a member of the Peptidase A1 family, and is the predominant digestive protease in the gastric juice of vertebrates. Pepsin has an approximate molecular mass of 34.6 kDa.[1]

Pepsin, unlike some other endopeptidases, hydrolyzes only peptide bonds. Pepsin does not hydrolyze non-peptide amide or ester linkages. Pepsin exhibits preferential cleavage for hydrophobic, preferably aromatic, residues in P1 and P1′ positions. Increased susceptibility to hydrolysis occurs if a sulfur-containing amino acid is close to a peptide bond which has an aromatic amino acid.

Pepsin will also preferentially cleave at the carboxyl side of Phe and Leu, and to a lesser extent at the carboxyl side of Glu residues. Pepsin will not cleave at Val, Ala, or Gly linkages.[2] Amidation of the C-terminal carboxyl group prevents hydrolysis by pepsin.[2,3]

Pepsin is commonly used in the preparation of $F(ab')_2$ fragments from antibodies. Pepsin-agarose has been likewise used in $F(ab')_2$ fragment preparation.[4,5]

This pepsin-agarose product is prepared by the immobilization of pepsin, originally isolated from porcine gastric mucosa, to activated 4% crosslinked beaded agarose. Several references have cited use of this specific product in various applications, including:

- Purification of stable isotope-labeled collagen[6]
- Analysis of distillers dried grains[7]
- Analysis of bakery meal dry matter[8]
- Digestion of prolamin proteins in coeliac disease biopsy samples[9]
- Simulating gastric digestion[10]
- Study of the peanut allergen Ara h 1 in Brown Norway rats[11]

Pepsin is generally most effective at acidic pH values, in the range of pH 1.5–2.0.[12] This pepsin-agarose product has been used at various acidic pH values, such as pH 2.5,[11] pH 3.2,[4] and pH 4.0.[5] Use of pepsin and pepsin-agarose should be avoided at pH >6, because of the risk of inactivation of pepsin above this pH. Pepsin is known to be irreversibly inactivated at pH 8, for example.[12]

Components

This pepsin-agarose product is sold as a lyophilized powder, with lactose present as a stabilizing agent.

Preparation Instructions

General instructions for re-suspension of the enzyme-agarose conjugates include the following steps:

1. Suspend the lyophilized enzyme-agarose to 5-10 mg solid/mL of water.
2. Allow brief hydration of the lyophilized powder.
3. Filter and wash the rehydrated enzyme-agarose product several times with either water or buffer of choice.
4. Re-suspend the enzyme-agarose in the buffer of choice. The product is now ready for use.

Storage/Stability

For re-use of the enzyme-agarose conjugates, the following steps may be used as a general guide.

- Wash the enzyme-agarose with water and/or buffer, such as 50 mM trisodium citrate (pH 5.0) specifically for pepsin-agarose,[13] until it is free of substrates.
- One publication has stored re-suspended pepsin-agarose in 10 mM sodium acetate buffer (pH 4.5), with 0.02% sodium azide as a preservative.[14] However, Sigma-Aldrich has not tested this specific situation ourselves.

- For long-term storage, enzyme-agarose products may be re-converted to their dry form, as follows:
  1. Wash the enzyme-agarose with 50 mM trisodium citrate (pH 5.0).
  2. Drain excess buffer.
  3. Dry the enzyme-agarose in a vacuum desiccator.
  4. Store the freshly lyophilized enzyme-agarose at 2–8 °C.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

References

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