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