

## Product Information

## Protease Inhibitor Cocktail

For plant cell and tissue extracts, DMSO solution

**P9599**

### Product Description

Crude cell extracts contain various endogenous enzymes, such as proteases and phosphatases, which can degrade proteins in the extracts. The best way to increase the yield of intact proteins is to add inhibitors of those enzymes known to be present.

The P9599 protease inhibitor cocktail has been optimized and tested for plant cell and tissue extracts. P9599 is a mixture of water-soluble protease inhibitors with a broad specificity for the inhibition of serine, cysteine, acid, and metalloproteases, as well as aminopeptidases.

P9599 is supplied as a solution in DMSO. The inhibitors in P9599 are as follows, with respective specific inhibitor targets and target classes of each inhibitor listed:

- AEBSF [4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride]: serine proteases, such as trypsin, chymotrypsin, plasmin, kallikrein and thrombin
- Bestatin hydrochloride: aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase<sup>1-4</sup>
- E-64 [*N*-(trans-Epoxy succinyl)-L-leucine 4-guanidinobutylamide]: cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L
- Leupeptin hemisulfate salt: serine proteases and cysteine proteases, such as plasmin, trypsin, papain, and cathepsin B
- Pepstatin A - acid proteases (such as pepsin, renin and cathepsin D) and many microbial aspartic proteases
- 1,10-phenanthroline: metalloproteases

Several theses<sup>5-14</sup> and dissertations<sup>15-30</sup> have cited use of product P9599 in their protocols.

### Storage/Stability

Store the cocktail at -20 °C. The product, as supplied, is stable for 4 years when stored at -20 °C, 8 months at 2-8 °C, and 2 months at room temperature.

### 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.

### Recommended Usage

One mL of the P9599 solution is recommended for the inhibition of endogenous enzymes found in 100 mL of lysate from 30 g (wet weight) of various plant tissues.

Whole extracts of plant seedlings from pea (*Pisum sativum*), bean (*Phaseolus vulgaris*), wheat (*Triticum aestivum*), tobacco (*Nicotiana tabacum*), and arabidopsis (*Arabidopsis thaliana*) have been tested. Extracts of leaves or roots from pea, wheat, and tobacco have also been tested. Please see the "Endogenous protease activities in plant tissues and their inhibition" section (Figures 1-5) elsewhere in this document.

**Note:** Not all lysates contain the same levels of endogenous enzymes. It may be necessary to adjust the volume of cocktail required.

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## Endogenous protease activities in plant tissues and their inhibition

Figure 1.

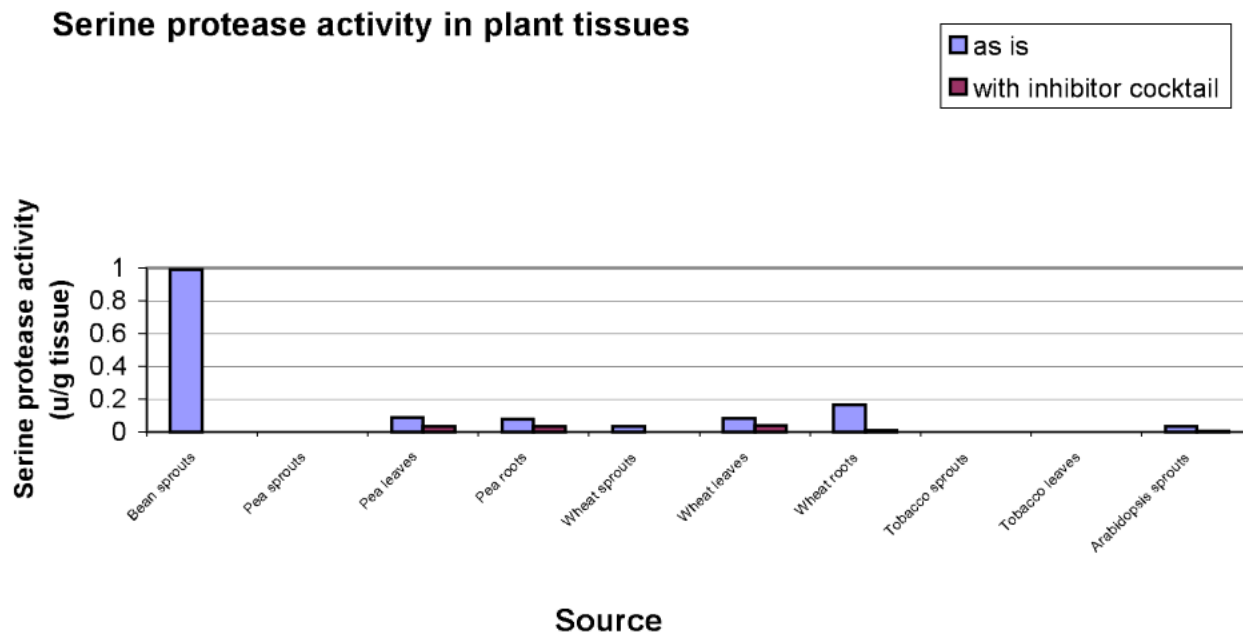


Figure 2.

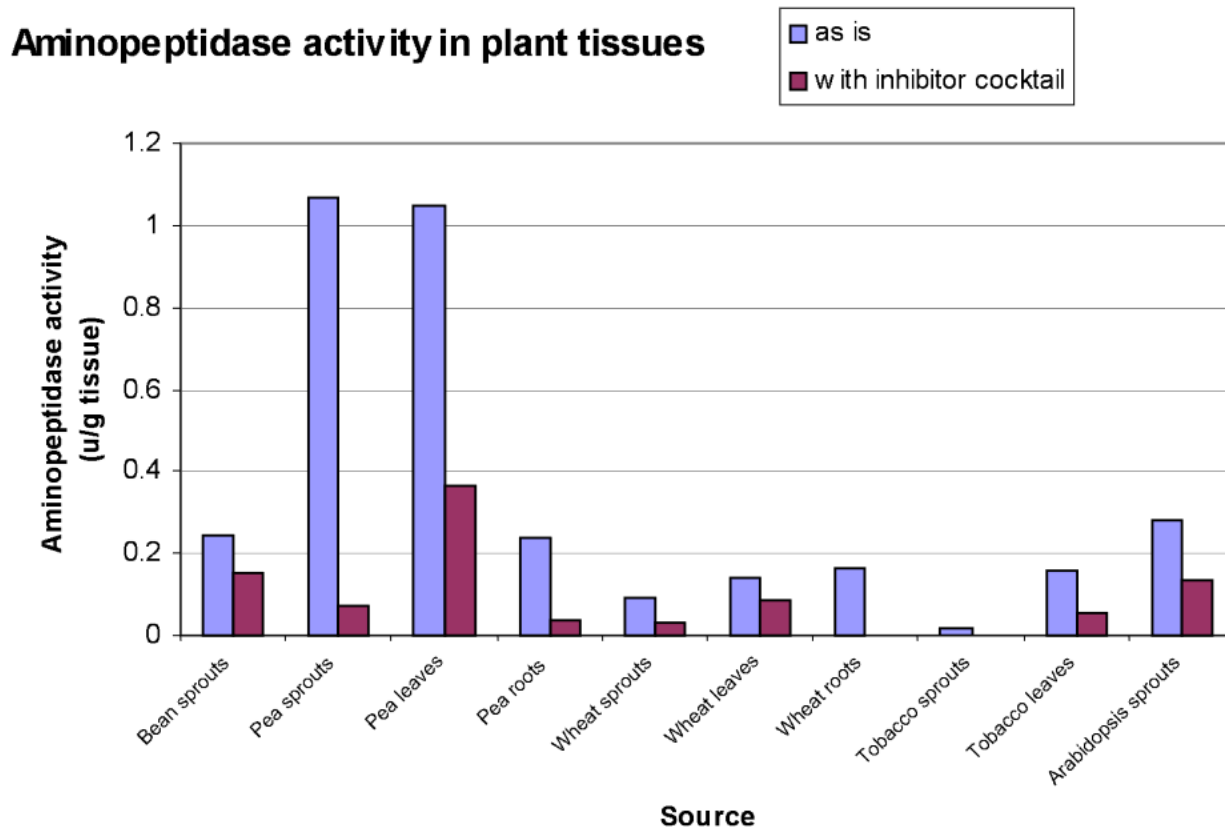




Figure 3.

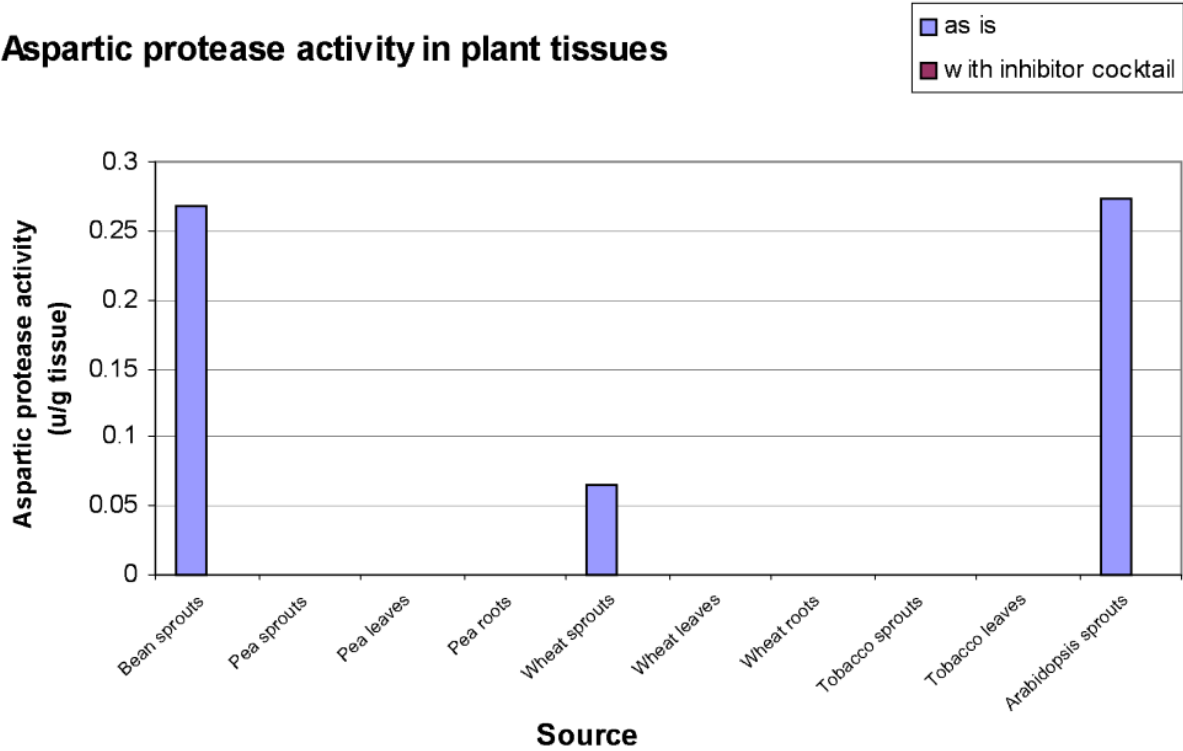


Figure 4.

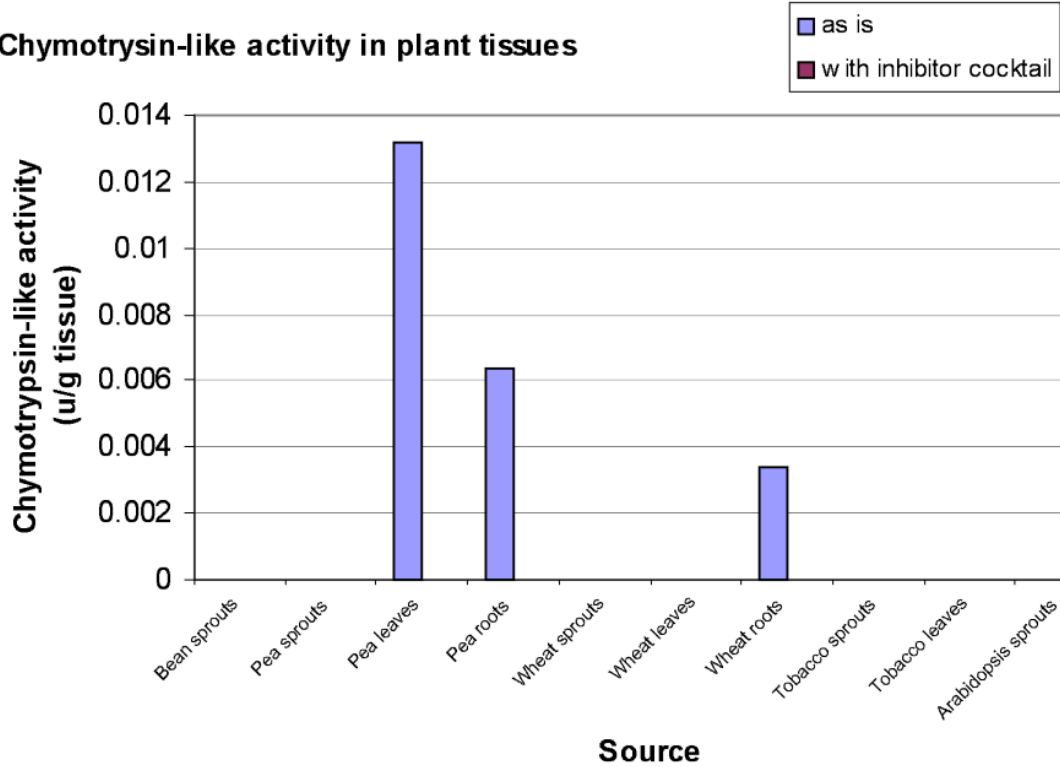
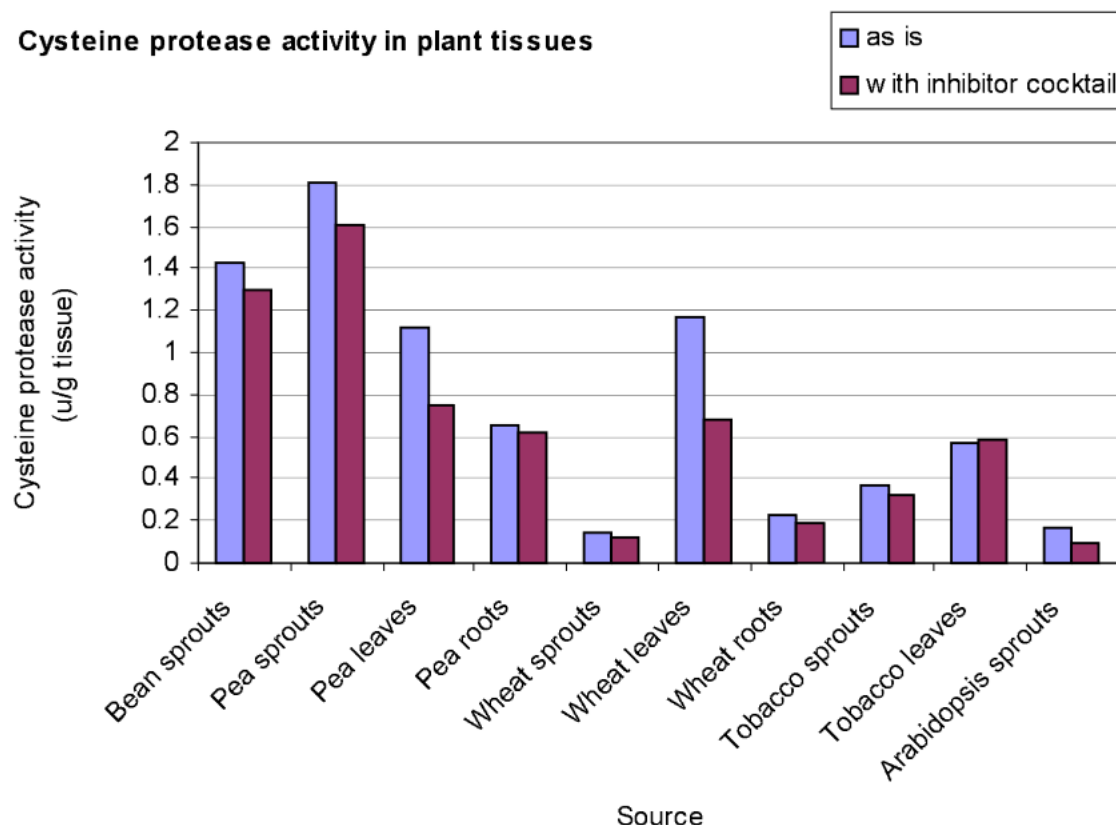


Figure 5.

### Cysteine protease activity in plant tissues



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