

## ProteaseArrest™ Protease Inhibitor Cocktail Offers Over 95% Inhibition of Proteases & Outperforms Tablet Format Cocktails

ProteaseArrest™ protease inhibitor cocktails contain optimized concentrations of various protease inhibitors, which provide excellent inhibition of protease activities during protein purification from various species.

ProteaseArrest™ cocktails inhibit a wide variety of protease classes, including serine and cysteine proteases. The optimized concentrations make the cocktails highly versatile, providing consistently high levels of inhibition.

ProteaseArrest™ inhibits 95% of all proteases in a wide variety of tissues, including mammalian tissues with high protease activity (i.e. pancreas).

ProteaseArrest™ cocktails have a greater efficiency of inhibition in high protease concentrations compared to tablet format protease cocktails and therefore offer superior protection of proteins during purification.

### AIM

To evaluate the protease inhibitor levels of ProteaseArrest™ in tissues with high protease activity and subsequently compare the degree of protease inhibition between ProteaseArrest™ and a leading, commercially available tablet form protease inhibitor cocktail in lysates with high proteolytic activity.

### METHOD

To determine the biological sample with high protease activities 0.5mg/ml lysates of mouse pancreas, kidney, liver, spleen and heart, and human Jurkat cells and spinach were screened with our Protease Screening Kit.

To compare the protease inhibition of ProteaseArrest™ and the tablet cocktail, triplicate reactions for a "Blank", "Control", "ProteaseArrest™" and "Tablet Cocktail" (Roche Complete™ EDTA-Free protease inhibitor cocktail tablet, Cat. #11873580001, Lot # 12037500) were prepared by aliquoting 10ml Protease Substrate and 10µl Incubation Buffer, supplied with the Protease Screening Kit, into 1.5ml tubes. In addition, 20µl sterile water was added to the "Blank" tubes and 20µl 0.5mg/ml mouse pancreas lysate to the "Control" tubes.

1µl reconstituted ProteaseArrest™ (EDTA-free) was added to 100µl 0.5mg/ml mouse pancreas lysate to give a final 1X concentration. 20µl of this lysate was added to the "ProteaseArrest™" tubes. The EDTA-free protease inhibitor cocktail tablet was prepared according to the manufacturer's instructions and added to 100µl 0.5mg/ml mouse pancreas lysate to give a final 1X concentration. 20µl of this lysate was added to the "Tablet Cocktail" tubes.

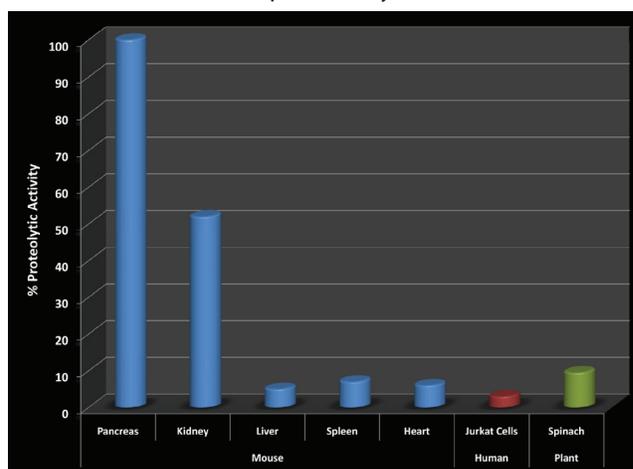
The samples were incubated for 2.5 hours at 37°C and the reaction was then stopped with the addition of 100µl

Precipitation Agent. Following centrifugation at 12,000g for 5 minutes, 80µl supernatant was transferred to tubes containing 120µl Assay Buffer and the absorbance was measured at 570nm.

The percentage inhibition was calculated with respect to the control samples.

### RESULTS & DISCUSSION

Figure 1 clearly demonstrates that the mouse pancreas has exceptionally high levels of protease activity compared to the other tissues screened and as a result the comparison of the protease inhibitor cocktails will be carried out with mouse pancreas lysates.



**Figure 1: Analysis of protease levels in a variety of lysates using Protease Screening Kit.** A variety of mouse lysates, human Jurkat cell lysate and spinach lysate were screened with G-Biosciences Protease Screening Kit to determine which lysate had the highest protease activity.

Figure 2 is a graphical representation of the results. The error bars were calculated from three separate experiments that utilized different lots of the protease cocktails.

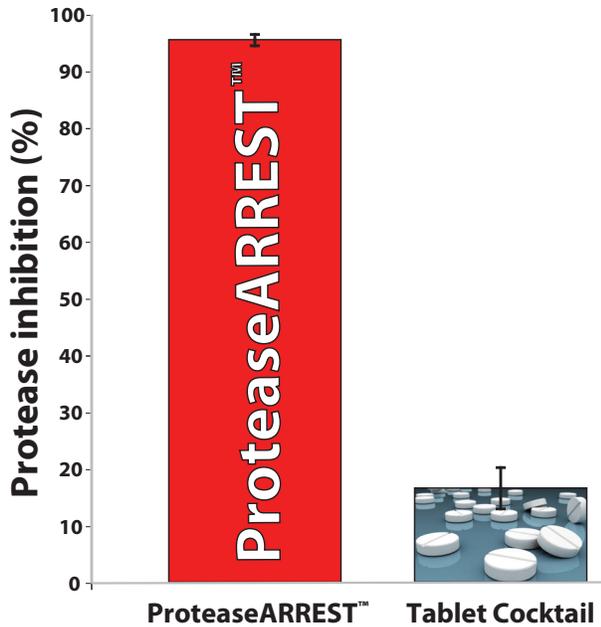
Figure 2 demonstrates that ProteaseArrest™ cocktail inhibits >95% of the proteases and has almost 80% more inhibition compared to a commercially available tablet format protease inhibitor cocktail.

Interestingly, the supplied tablet protease inhibitor cocktail literature reports inhibition of pancreas lysates of over 90%, however closer inspection shows two main differences between the assays used. The most significant difference is that the concentration of pancreas lysate was 25 times more dilute in the competitor's reported assay (0.02mg/ml as opposed to 0.5mg/ml), meaning 25 times less proteolytic activity. Secondly, the incubation conditions were 60 minutes at 15-25°C as opposed to 150 minutes at 37°C, suggesting that ProteaseArrest™ has greater stability with respect to time and temperature.



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**Figure 2: ProteaseArrest™ has high, reproducible protease inhibition.** Protease inhibition in mouse pancreas lysate with ProteaseArrest™ (EDTA-free) and a commercially available EDTA-free tablet protease cocktail was compared, using Protease Screening Kit. The assay used 0.5mg/ml pancreas lysate and incubation conditions of 37°C for 2.5 hours. ProteaseArrest™ inhibited over 95% of total proteases, almost 80% more protease inhibition compared to the competitor's tablet protease inhibitor cocktail.

So ProteaseArrest™ is more stable, has significantly greater inhibition, and offers greater protection against proteases during prolonged incubation and handling times.

In independent studies, researchers have found that ProteaseArrest™ outperforms several leading manufacturer's protease inhibitor cocktails, including tablet formats, in the purification of plant proteins (1).

ProteaseArrest™ is available with EDTA, to inhibit metalloproteases, or without EDTA, to maintain activity of proteins dependent on divalent cations. The EDTA-free ProteaseArrest™ will not inhibit the purification of proteins using immobilized metal affinity chromatography (IMAC).

ProteaseArrest™ is also available as single use aliquots that are suitable for >95% protease inhibition in 10ml solutions. These OneQuant™ ProteaseArrest™ (Cat. #786-329) are provided for additional protease inhibitor cocktail convenience.

The convenient format of ProteaseArrest™ ensures the delivery of consistent concentrations of protease inhibitors, that have a high protease inhibition (>95% in mouse pancreas lysate).

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**ORDERING INFORMATION**

Cat. #	Description/ Size
786-108	ProteaseArrest™ [100x]/ 2ml
786-437	ProteaseArrest™ [100x]/ 5ml
786-711	ProteaseArrest™ [100x]/ 10ml
786-712	ProteaseArrest™ [100x]/ 5 x 10ml
786-329	OneQuant™ ProteaseArrest™ [100X]/ 24 x 100µl



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