

## ***Bt* Toxin Degradation with E3 Advanced Plus**

### **Objective:**

To determine the degradation of *Bt* Toxin Protein with E3 Advanced Plus (LB16.885) and Pancreatin (LB16.1373) within 90 minutes of introduction.

### **Method:**

First, the following reagents were prepared:

- 0.15M 4.5 pH sodium acetate buffer, 500mL. Made with 10g sodium acetate anhydrous into 200mL DI H<sub>2</sub>O. Stirred until fully dissolved and lowered to pH 4.5 with 1N HCl. Diluted to 500mL with DI H<sub>2</sub>O.
- 1 ThermoFisher Silverstain Kit.

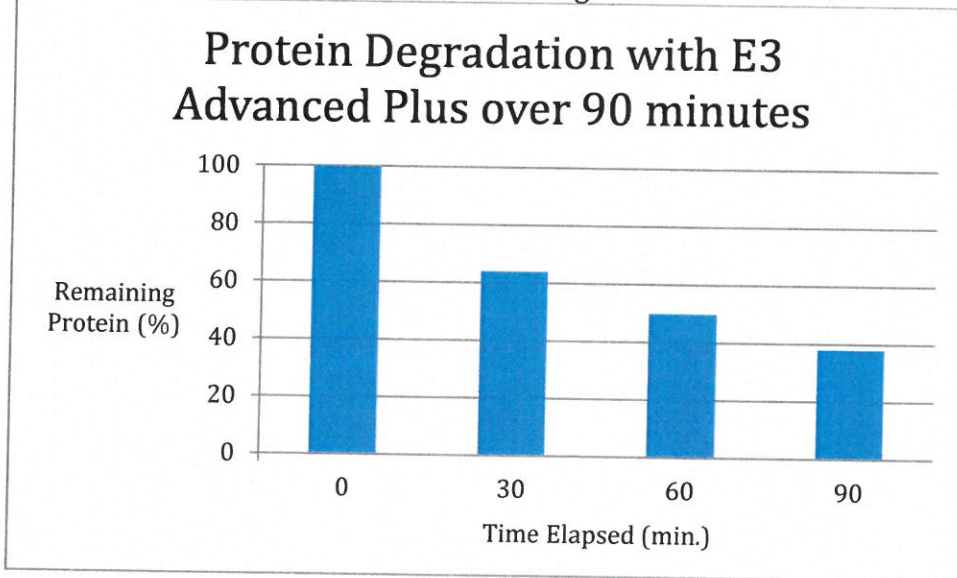
Next, 350mg E3 Advanced Plus was weighed out and diluted 1:100 twice in DI H<sub>2</sub>O, and vortexed. Additionally, 350mg Pancreatic Enzymes were weighed out, and diluted 1:100 twice in DI H<sub>2</sub>O, and vortexed. 5µL E3 Advanced Plus solution or Pancreatic Enzymes and 5µL *Bt* Toxin solution were placed into 90µL Sodium Phosphate Buffer 3 separate times, for the time 0, 30, 60, and 90 samples. This was done twice – once for the E3 Advanced Plus Solution, and once for the Pancreatic Enzymes. Samples were taken out of a 37°C waterbath at time 0, 30, 60, and 90 minutes, and boiled immediately for 5 minutes. After all samples had been collected and boiled, they were diluted 1:1 with 25µL of sample buffer and boiled again for 5 minutes. These finished samples were then loaded into the microwells of the prepared gel at 12µL each. The gel was then run at 200V using 1x running buffer until the dye front reached the bottom of the gel.

### **Materials Used:**

- 0.15M pH 4.5 pH sodium phosphate buffer
- 1x running buffer
- Silverstain Kit
- E3 Advanced Plus LB16.885
- Pancreatin LB16.1373
- Sample buffer 2X R15.056
- Gel R16.289

**Results:**

Fig. 1 – Densitometry Data for Protein Degradation



**Discussion:**

Densitometry of gel was determined using the National Institute of Health (NIH) ImageJ software. Using this information, it was concluded that in simulated intestinal conditions, E3 Advanced Plus and Pancreatin are each capable of digesting more than half of the present *Bt* Toxin within 90 minutes. This implies that when working in concert with the body's natural pancreatic enzymes, E3 Advanced Plus should reduce *Bt* protein levels greatly, to near-zero dosages.

For reference, the average *Bt*-treated crop contains safe levels of about 100-500 parts per million of the *Bt* protein [1]. Within 90 minutes of E3 Advanced Plus and Pancreatic digestion, levels could be as low as 0-60ppm – well below the EPA-approved acceptable levels of 5,000ppm.

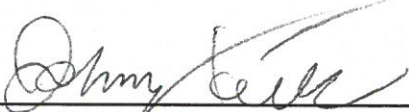
In conclusion, consumption of E3 Advanced Plus is likely to reduce *Bt* protein levels in *Bt*-treated crops to doses considerably smaller than those recognized as safe in mammalian organisms [2].




*From concept to commercialization, we add value at every step.\**

## References

1. U.S. Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division. Cry1Ab and Cry1F *Bacillus thuringiensis* (Bt) Corn Plant-Incorporated Protectants. 2010. Biopesticides Registration Action Document.
2. Mendelsohn M, Kough J, Vaituzis Z, Matthews K. Are Bt crops safe? 2003. Nature Biotechnology Vol. 21 #9.

  
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