



College of Agriculture  
School of Nutrition and Food Sciences

February 20, 2018

Mr. Drew Perrin  
CEO Ecovatec Solutions Inc.  
31231 Wheel Ave.,  
Abbotsford, BC, CANADA V2T 6H1

Re: Analysis of Ecovatec's Phosvitin

Dear Mr. Perrin,

I am pleased to report to you that, after extensive analysis of Ecovatec's phosvitin powder sample, I have determined that it is of **high quality**. I have a long history and considerable experience isolating and evaluating phosvitin. Early in my career, I developed a simple laboratory procedure, now widely adopted and cited by researchers, for the isolation of phosvitin from avian eggs or fish roes. Further, I have studied phosvitin for years, allowing me to state that I am an expert in phosvitin chemistry and biochemistry.


In many academic studies, researchers prove they have isolated pure phosvitin by comparing its gel electrophoresis profile and chemical composition to a sample "gold" standard from Sigma Aldrich. A positive match allows scientists to conduct experiments to illustrate the properties and applications of phosvitin and its peptides as a bioactive protein and peptides, respectively.

Ecovatec's sample of phosvitin shows the same subunit profile (the  $\alpha$ - and  $\beta$ - phosvitins) as Sigma Aldrich's under the denaturing conditions of gel electrophoresis. In these results, attached, phosvitin separates into its characteristic subunits of molecular weight ranging from 12,400 to 119,000 Daltons.

Phosvitin is the most highly phosphorylated protein found in nature and serine composes 50% of the amino acids of phosvitin. Ecovatec's phosvitin has 49.8% serine, showing that the sample is of **high quality**. Ecovatec's phosvitin has 12.8% nitrogen, 80% protein (by Dumas) and 1,604-2,335.35 ppm phosphorus (determined by ICP) in the powder. Sigma's phosvitin is 70% protein (as reported by Sigma) and has 691.6 ppm of phosphorus (by ICP). I also prepared phosvitin using the protocols developed by Ko et al. in 2010 and Losso and Nakai in 1994, which are widely used in the scientific literature, to compare to yours. The gel electrophoretic profiles of the laboratory-isolated phosvitin provided the same result as Ecovatec's phosvitin. This allows me to conclude that **Ecovatec's phosvitin is of high quality**.

Phosvitin isolation at the commercial level has been a challenge to many. As a result, there is no phosvitin for large scale usage due to its cost. Ecovatec's technology to provide commercial quantities of phosvitin to users around the world is a **breakthrough that will fulfill the needs of the food and biomedical industries**. I look forward to investigating Ecovatec's phosvitin in my research. If I can be of any assistance in the near future, please feel free to contact me.

Thank you for your consideration,

  
Jack Losso, Ph.D.; CFS.