



ecovatec
SOLUTIONS INC

Inspired Functional Ingredients to Innovate and Differentiate Your Brand

Products by Ecovatec Solutions Inc.

Product Background



- Important to understand the term “bioactivity”
- Scientists are trying to find “bioactive peptides”
- “Able to exert a biological effect at a physiological level, must be measurable and able to provide health benefits.”
- Bioactive molecules show promising:
 - nutraceutical,
 - pharmaceutical,
 - tissue engineering, and
 - cosmetic effects



ecovatec
SOLUTIONS INC

Phosvitin (PV)



EcovaPure™ PV



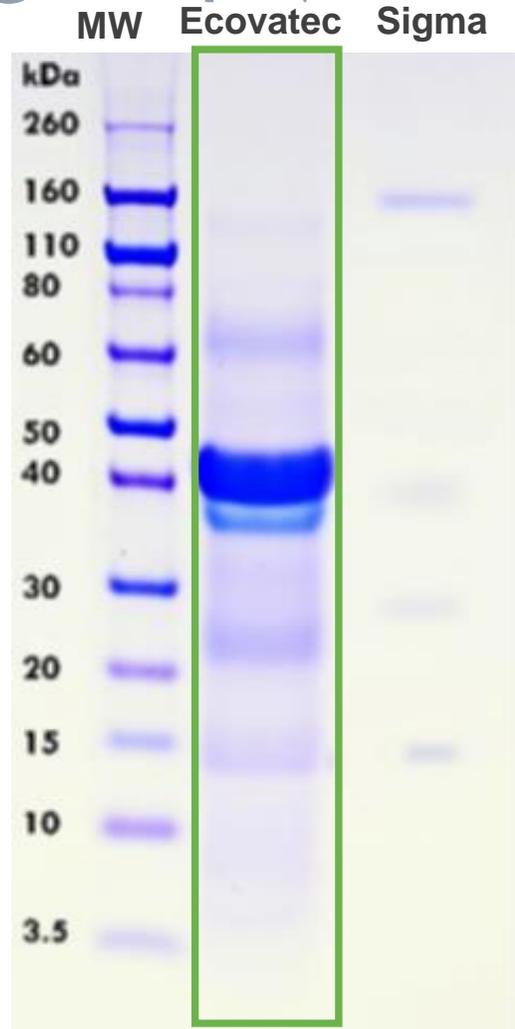
Phosvitin Background

- Scientists have been isolating and researching phosvitin's properties for decades.
- PV has been well characterized by the scientific literature and is known to have many therapeutic applications.
- PV is considered to have extensive bioactive properties and its applications have only been hampered by a lack of availability.
- Since phosvitin is extremely expensive and only available from Sigma Aldrich, scientists who research it prove they have phosvitin by isolating it in the lab and comparing its "Gel Electrophoresis Profile" (GEP) to the "standard" from Sigma Aldrich
- Ecovatec is the **first** and **only** commercial producer of phosvitin.

EcovaPure™ PV



Like researchers,
Ecovatec has
proven that it has
produced
phosvitin by
comparing our
sample to the
Sigma Aldrich
standard



Phosvitin

(Phosvitin is present at these
molecular weights).

Pure Phosvitin protein has
50% serine. Ecovatec
phosvitin has 49.2% serine.

EcovaPure™ PV



You can also compare our Certificates of Analysis to those of the “Sigma Aldrich Standard”

SIGMA-ALDRICH™

3350 Spruce Street, St. Louis, MO 63103, USA
 Website: www.sigmaaldrich.com
 Email USA: info@sigmaaldrich.com
 Outside USA: outsideusa@sigmaaldrich.com

Certificate of Analysis

Product Name: Phosvitin from egg yolk
 Product Number: P1250
 Batch Number: SLDG2021V
 Brand: SIGMA
 CAS Number: 9206-96-2
 MDL Number: MFCD00121903
 Storage Temperature: Room at 20-25 °C
 Quality Release Date: 01 SEP 2012

Test	Specification	Result
Appearance (Color)	White to Light Yellow	Off-White
Appearance (Form)	Powder	Powder
Solubility (Color)	Risk Yellow to Dark Yellow	Yellow
Solubility (Turbidity)	Clear to Very Slightly Hazy	Very Slightly Hazy
50 mg/mL H ₂ O		
Water (by Karl Fischer)	≤ 10 %	5 %
Phosphorus (P) (anhydrous)	8 - 12 %	9 %
Nitrogen (N) (anhydrous)	10.2 - 11.2 %	11.2 %

Rodney Burdock, Manager
 Analytical Services
 St. Louis, Missouri, US

Sigma-Aldrich warrants that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of this product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

Page 1 of 1

Certificate of Analysis

Product Name:

Phosvitin

Batch Number: 198201
 Manufacturer: ECOVATEC, INC
 CAS Number: 9206-96-2
 Formula: N/A
 Storage Temperature: -20 °C

Test	Specification	Result
Appearance (Color)	White	White
Appearance (Form)	Powder	Powder
Solubility (Color)	Colorless to Very Faint Yellow	Very Faint Yellow
Solubility (Turbidity)	Clear	Clear
50 mg/mL in water (at 30 °C)		
Phosphorus (P) (anhydrous)	8-10%	8.2%
Nitrogen (N) (anhydrous)		12.8 %
GCMS		49.2%

Jack Luzzo, Ph.D.; DFG
 Professor
 Louisiana State University
 Baton Rouge, Louisiana, US

Sample	N (%)	Protein (%)
EcovaPure PV	12.8	80
Sigma	11.2	70

EcovaPure™ PV

A letter from Jack Losso Ph.D.; CFS confirming our phosvitin's quality



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.

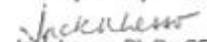
In many academic studies, researchers prove they have isolated pure phosvitin by comparing its gel electrophoresis profile and chemical composition to a sample "gold" standard from Sigma Aldrich. A positive match allows scientists to conduct experiments to illustrate the properties and applications of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

Ecovatec's sample of phosvitin shows the same subunit profile (the α - and β -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.

EcovaPure™ PV



Phosvitin Properties

- Ecovatec is the **first and only** commercial phosvitin producer
- Patent pending on Ecovatec's proprietary extraction technique
- Current market price > USD \$3M / kg for research quantities
 - available @ Sigma Aldrich
<https://www.sigmaaldrich.com/catalog/product/sigma/p1253>
- Phosvitin is the MOST phosphorylated protein in nature
 - Phosvitin 123 phosphoserine groups
 - compared to Caseins <15 phosphoserine groups
 - Gives it unique “bioactive” properties



ecovatec
SOLUTIONS INC

Phosvitin Phosphopeptides (PVP)

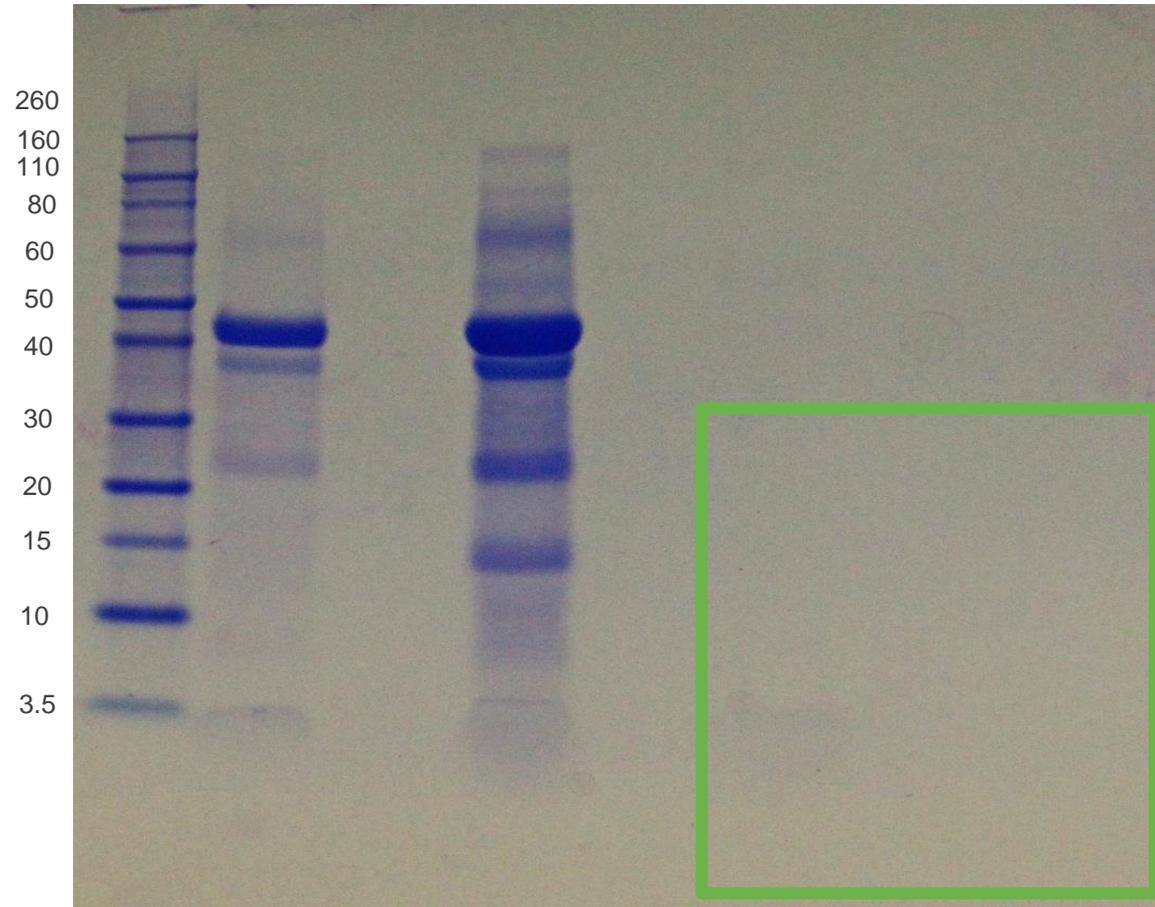


EcovaPure™ PVP



ecovatec
SOLUTIONS INC

MW 601 PV Lab PVP 301 PV Lab PVP **101PVP** 601 PVP 301PVP 301 PVP

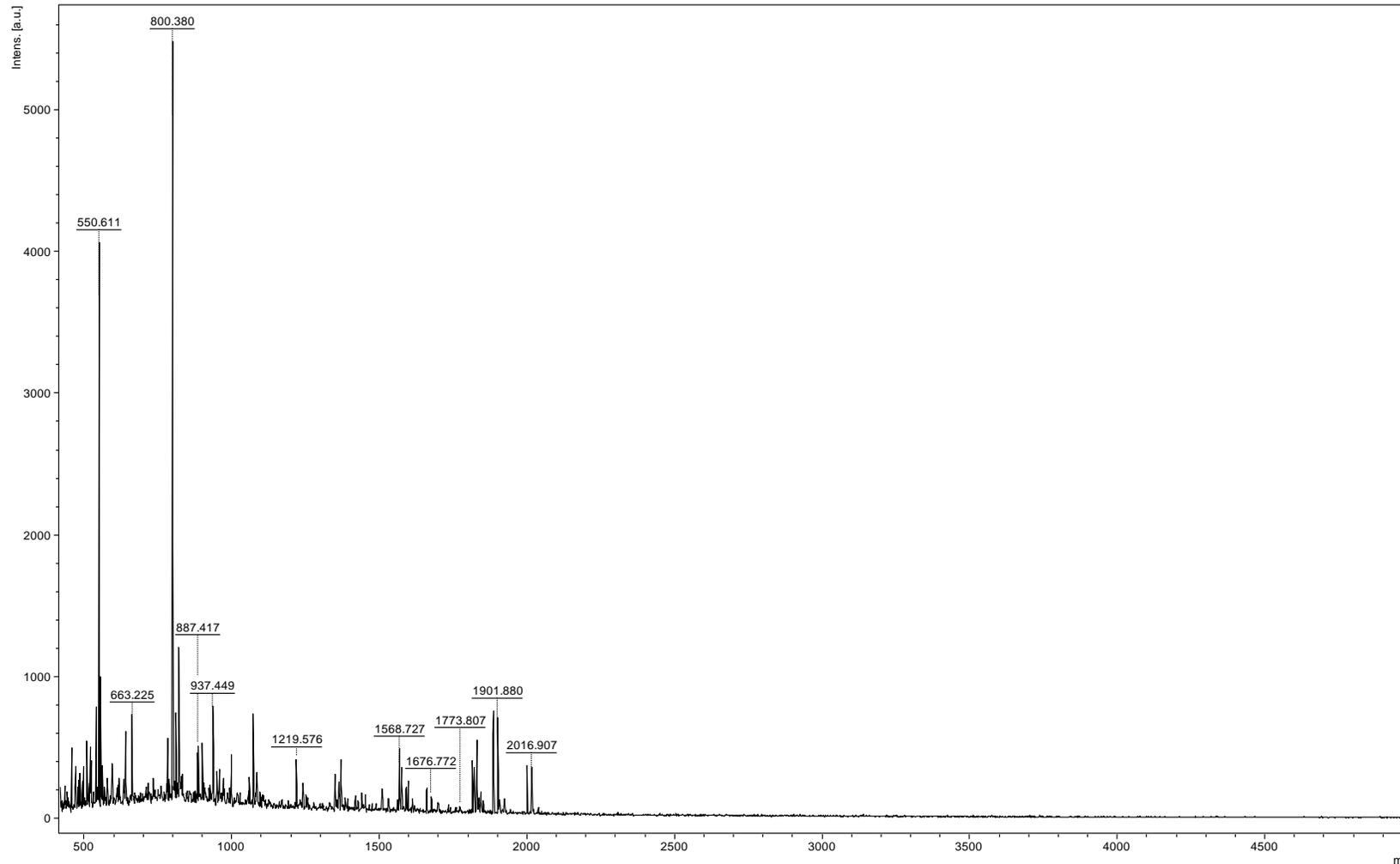


Lack of banding
shows that the protein
was effectively
hydrolyzed into small
peptides

EcovaPure™ PVP



ecovatec
SOLUTIONS INC



All proteins broken down to under 2.1 kDa with most peptides < 1 kDa (0.4- 5 kDa range shown)

A second MALDI of the 5 -15 kDa range shows that there were no peptides in that range either. (not shown here)

EcovaPure™ PV & PVP



Phosvitin Phosphopeptides (PVP)

- Phosvitin (PV) can be hydrolyzed into its smaller peptides which increases most of its bioactive properties.
- Ecovatec performs this processing to create PVP without using any harmful solvents or high temperatures/pressures to ensure that the peptides retain their full potential.
- Extensive research has been performed on phosvitin and its phosphopeptides and the applications for the pharmaceutical, nutraceutical, and cosmetic industries.



EcovaPure™ PV & PVP



ecovatec
SOLUTIONS INC

PV and PVP's Bioactive Functions:

- Increases iron and calcium absorption
- Acts as anti-oxidant, Anti-bacterial, Anti-viral
- Anti-cancer
- Toxin-neutralizing
- Inhibits melanogenesis

EcovaPure™ PV & PVP



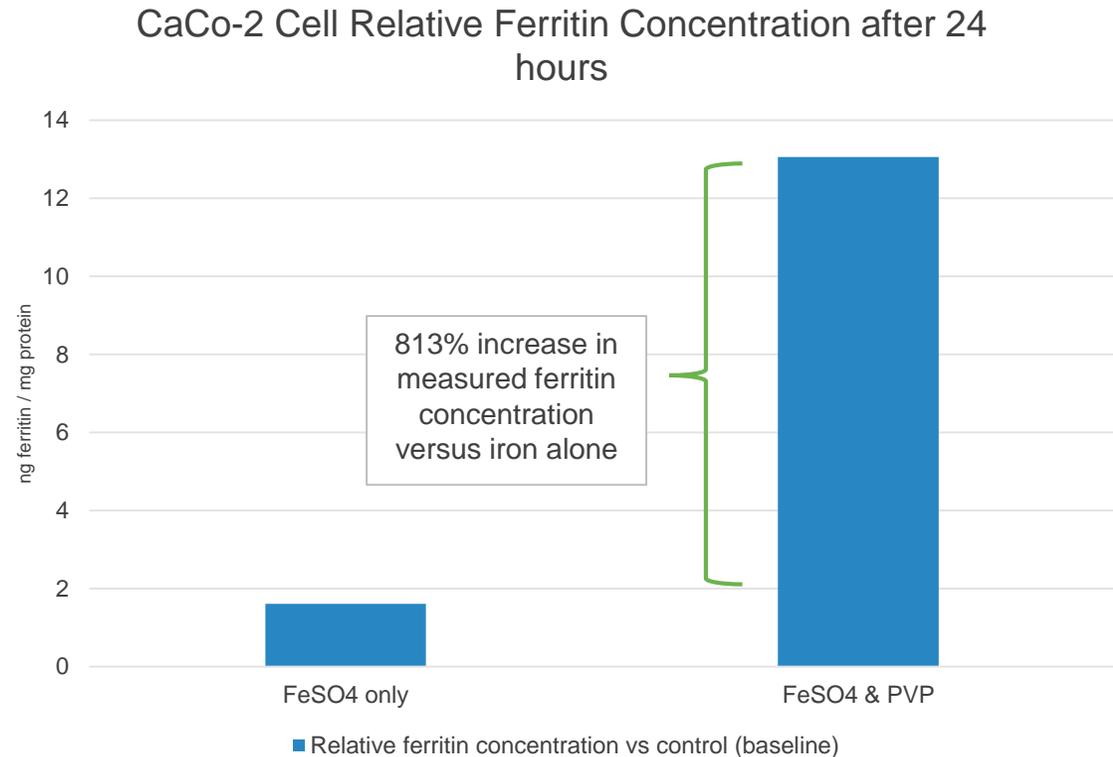
Increases iron absorption:

- Iron deficiency affects 4-5 billion people worldwide (WHO)
- Supplements have gastrointestinal side effects due to high doses of iron, which are required due to very poor bioavailability
- PVP is an excellent metal chelator (binder) and was tested to see if bioavailability was improved
- Feng and Mine found PVP increased iron uptake in human intestinal cell model by over 3x

EcovaPure™ PV & PVP



- Ecovatec's Caco-2 experiment preliminary results show an **8x improvement** in Caco-2 cell ferritin levels over iron sulfate alone
- A formulated product with this bioavailability would allow for lower iron dosages and the elimination of side effects!



EcovaPure™ PV & PVP



ecovatec
SOLUTIONS INC

Increases calcium absorption:

- Low calcium disorders: osteoporosis, low bone density
- Higher calcium linked to preventing: colorectal cancer, cardiovascular disease, high blood pressure, preeclampsia
- Higher calcium linked to promoting weight management
- Rat studies showed when diets enriched with PVP, calcium in bones increased 10-20% (in the presence of 160 times more casein)
- PVP helps converts some calcium to HAP in the gut. When patients consumed HAP as supplement, slowed bone loss

EcovaPure™ PV & PVP



ecovatec
SOLUTIONS INC

Acts as anti-oxidant:

- In the body, free radicals are produced daily. They cause oxidative stress.
- Oxidative stress is associated with: cardiovascular diseases, ageing, neurological disorders (Alzheimer's, Parkinson's), inflammatory diseases, diabetes, and more.
- Lipid oxidation in food products like beef are associated with reduced shelf life and quality.
- Studies show the effectiveness of PV in food processing and PVP in inhibiting enzymes associated with type II diabetes, which could help patients maintain stable blood sugar.

EcovaPure™ PV & PVP



Inhibits melanogenesis:

- Humans have a “base level” of melanin. When exposed to UV rays, they have an “activated level”
- Excess melanin can be associated with aesthetic problems like hyperpigmentation and age spots. But these can also be associated with increased risk of skin cancer.
- Phosvitin (PV) shown to significantly inhibit the production of melanin in skin cancer cells, suggesting potential therapeutic use in creams and lotions.

EcovaPure™ PV & PVP



ecovatec
SOLUTIONS INC

Anti-bacterial:

- Antibiotics are resulting in drug-resistant bacteria.
- Researchers are investigating anti-bacterial peptides for new treatment.
- PVP (and other anti-bacterial peptides) acts on the bacteria through multiple mechanisms, causing bacteria cell death.
- Current anti-bacterial peptides are toxic to human cells.
- PVP is not toxic to human cells and are effective on multi-drug resistant bacteria.
- It is also effective against endotoxin mediated sepsis, which currently has no effective drug and 50% patient mortality.

EcovaPure™ PV & PVP



ecovatec
SOLUTIONS INC

Anti-cancer:

- Phosvitin investigated in 7 different human cancer cell lines *in vitro*.
- PV was 95% effective at killing cervical cancer cells; slowed growth of breast cancer cells by 94%.
- Effects most pronounced in killing liver cancer cells.
- Was also effective against stomach cancer, lung cancer, and larynx cancer.
- Huge therapeutic potential with further pharmaceutical and medical research.

EcovaPure™ PV & PVP



ecovatec
SOLUTIONS INC

Anti-viral:

- Being investigated first in marine and freshwater fish.
- A peptide isolated from native phosvitin protected against a major fish virus.
- Many antimicrobial peptides may also act as antiviral peptides.
- Needs further research to extrapolate to humans, but research has been hampered due to cost of phosvitin phosphopeptides.

EcovaPure™ PV & PVP



ecovatec
SOLUTIONS INC

Preservative Applications:

- Currently under study

EcovaPure™ PV & PVP



Summary:

Area of Application	Description
Nutraceutical	Iron and calcium supplements, antioxidant supplement for heart, brain, and general health, anti-inflammatory
Food Industry	Preservative for extended shelf life
Cosmetics	Reduces the appearance of hyperpigmentation and age spots due to blockage of melanin. Potential skin cancer prevention. Can be used as a natural cosmetics preservative
Pharmaceutical	Potential for anti-bacterial, anti-cancer, and anti-viral pharmaceuticals.



ecovatec
SOLUTIONS INC

Inspired Functional Ingredients to Innovate and Differentiate Your Brand

Products by Ecovatec Solutions Inc.