

## INTRODUCTION

Keyhole Limpet Hemocyanin (KLH) is a complex, oxygen-transporting protein. The sole source for KLH is the hemolymph of the marine mollusk *Megathura crenulata* (the Giant Keyhole Limpet) which exists in small wild populations in only one natural locale.

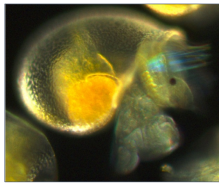
KLH is a safe, potent stimulator of humoral and cellular immune responses and, as such, has played a significant part in development of new immunotherapies and immunomodulating drugs for over fifty years. As an active ingredient, KLH is often used as an immune-stimulating carrier molecule in disease-targeting vaccines. As an injectable product, KLH has a vital role in research and clinical studies as an antigen for monitoring the immunosuppressive effects of new drug candidates.

Rising need for KLH by the pharmaceutical industry brought commercial attention to unsustainable fishery practices and mounting constraints on clinical supply of the vital KLH protein.

Our work in aquaculture science, specifically directed to the conservation of the Giant Keyhole Limpet and to non-harmful extraction of KLH protein, are proving successful in averting depletion of the wild source species *M. crenulata* as well as providing a commercial-scale system capable of ensuring KLH protein that meets the pharmaceutical industry's high standards.

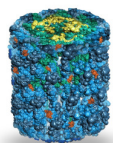
## AMAZING GIANT KEYHOLE LIMPET

The Giant Keyhole Limpet (*M. crenulata*) is a scarce marine mollusk and the sole source for KLH protein. It naturally lives in the rocky shallows only along a limited stretch of Pacific coastal waters, from Monterey Bay, California to Isla Ascension off Baja California, Mexico.



*Megathura crenulata*  
Mature Adult (left) and Veliger  
Larvae Stage (above)

Native KLH protein is extremely large and complex, with subunit isoforms assembled into decamers and didecamers of 4MDa to 8 MDa mol. wt. Two subunit isoforms (~360-400 kDa monomeric mol. wt.) are composed of 7 or 8 functional units, each containing an oxygen-binding site of two copper atoms.



KLH Structure Showing Complexity of  
160 Functional Units.

Different forms of KLH have different structural properties and effects on the immune system. The processing of KLH can impact isoform structure and immunogenicity. Recent preclinical data shows that commercial source and form of KLH can impact immunogenicity.

Attempts to synthesize KLH have been unsuccessful due to the molecule's large size and unusual glycosylation. KLH can only be prepared by purification from the hemolymph of the source animal, putting significant ecological pressure on the limited wild populations.

## FROM OCEAN TO IMMUNOTHERAPY

The robust immunogenicity and excellent safety record of KLH make this molecule a popular antigen of choice for a range of therapeutic and diagnostic immunological applications.

### KLH Therapeutic Applications

Historically, hemocyanins such as KLH were primarily used in research applications where only small quantities of non-GMP material sufficed. Since the 1990's, KLH has been increasingly used as an immune-stimulating carrier protein in active immunotherapies (a.k.a. therapeutic vaccines) being developed for treatment of cancers (breast, lung, ovarian, prostate, brain, and melanoma), systemic lupus erythematosus, rheumatoid arthritis, Crohn's and Alzheimer's.



KLH Solution in Commercial  
Vial Packaging

The small haptens used to target these diseases are not usually immunogenic enough to awaken the body's immune system on their own. They require a carrier protein and/or adjuvant to stimulate antibody production. KLH is effective in this role due to its natural immune-stimulating properties, large size, numerous sites for antigen conjugation, and safety profile.

### KLH Diagnostic Applications

The T-cell dependent antibody response (TDAR) test is a functional assay used in immunotoxicology to measure the effects of drug candidates such as the new classes of immunomodulating drugs in development pipelines. KLH is used extensively as an injectable immunogen in TDAR testing to stimulate antibody response (IgM and/or IgG) to immunization which can be measured via standard assay platform such as ELISA. TDAR testing with KLH is designated by regulatory agencies as standard method to evaluate immunotoxicity of investigational drugs.

### Impact: Rising Demand

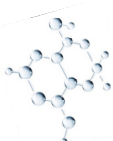
Factors impacting the demand for pharmaceutical quality KLH:

- Recent shift in drug development toward treatment strategies that focus on deploying the body's immune system to target disease;
- Growing incorporation of KLH as an active ingredient in certain active immunotherapies; and
- Regulatory requirements to monitor the effects of immunomodulating drugs by using a demonstrated-safe antigen to measure TDAR.

### Clinical Trials of KLH-Based Immunotherapies\*

Institute/Company	Indication	Clinical Phase
Affiris/GlaxoSmithKline	Alzheimer's	II
Bayer	N-H Lymphoma	I
Baylor Research	Melanoma	I/II
Biovest	N-H Lymphoma	MAA
Celldex	Glioblastoma	III
Loyola University	Ovarian Cancer	II
MabVax	Sarcoma	II
NCI	Leukemia	I/II
Obi Pharma	Breast Cancer	III
Neovacs	Crohn's, Lupus, RA	II
Sloan-Kettering	Lung Cancer	I

\* Not a comprehensive listing.  
Source: NIH ClinicalTrials.gov/2014



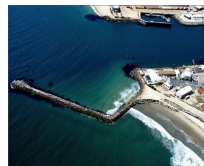
## ADVANCES IN AQUACULTURE & KLH PRODUCTION

We spent the past ten years advancing aquaculture science and KLH production, with the dual aim of meeting pharmaceutical industry demand and standards for KLH, while protecting the Giant Keyhole Limpet.

Result: Commercial-scale, land-based aquaculture systems with sustainable production capacity, which are not reliant on sensitive wild populations of *M. crenulata*.

### Unique Land-Based Aquaculture Facilities

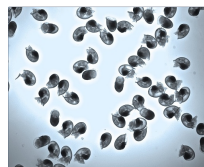
Stellar Biotechnologies developed and optimized methods to control reproduction and growth of *M. crenulata* including culture systems, nutritional requirements, seawater purification and induction of spawning. The team was successful in developing hatchery and nursery facilities that effectively control all phases of the mollusk lifecycle and optimize production as evidenced by (1) first-ever industry achievement of multiple generations of *M. crenulata*, grown entirely within our facility, producing KLH; and (2) scalable KLH production capacity.



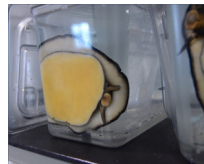
World's Only Land-Based Aquaculture Facility for *Megathura crenulata*  
Stellar Biotechnologies (Port Hueneme, CA)

### Successful Protein Extraction and Purification

By establishing comparability and uniformity of KLH isotype distribution between wild-capture and aquaculture, and through development of non-harmful extraction of KLH hemolymph from controlled/cultured colonies, we developed unprecedented controls related to lot traceability, quality, consistency and immune-stimulating performance; benefits valued by vaccine manufacturers using KLH. This paves the way for long-term, scalable supply of GMP grade KLH without putting wild populations of *M. crenulata* at risk.



Keyhole Limpet Larvae in Nursery (above left), Handful of Juveniles (right),  
Adult Limpet (bottom left), and KLH in QC Labs (below right)



The KLH molecule is extracted from Giant Keyhole Limpet hemolymph. Depending on product formula and grade, the protein is processed, purified and packaged into various commercial configurations.

## SUMMARY

- KLH protein: An important pharmaceutical industry product**
  - Robust immune-stimulating properties, strong safety profile
  - 50+ years as vaccine carrier molecule & assay immunogen
  - Rising demand
- One source**
  - KLH can only be obtained from the hemolymph of the scarce sea mollusk Giant Keyhole Limpet (*Megathura crenulata*)
- One location**
  - Megathura crenulata* is only native to the Pacific Ocean coastline of California and Baja California
- Reliance on sensitive wild populations is not ecologically sustainable and cannot keep pace with increasing pharma industry needs and standards**
- Advances in aquaculture & KLH production can both protect a species and supply growing commercial demand**
  - World's only demonstrated aquaculture systems sustaining full lifecycle
  - Non-harmful protein extraction methods
  - Full traceability from protein lot to source
  - Optimized manufacturing control and yield

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