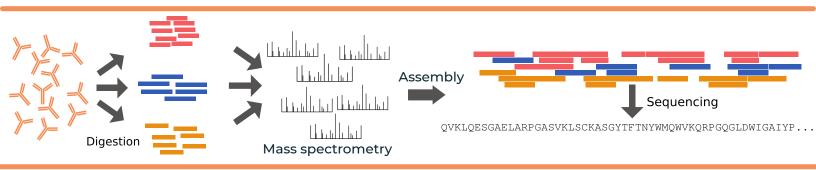


# **Antibody Protein Sequencing**

Lost your hybridoma? Want to convert to a recombinant monoclonal antibody? Valens is an antibody protein sequencing service that uses mass spectrometry and proprietary analysis software.

# The Valens<sup>TM</sup> Workflow



## **Key Features:**



Any Species
Human, mouse, rat, hamster, rabbit. and llama antibodies.



**Any Isotype** IgG1, IgG2, IgM and others.



Celebrating
Over 10 Years Of Sequencing!

### **Deliverables:**

- Full-length sequences of heavy and light chain
- Free Leucine and Isoleucine determination with CLIP.
- Interactive HTML report
- Recombinant expression and binding validation (Optional)

Starting Material 100ug, >90% purity

Turnaround Time
3 weeks

All work performed in the USA!



#### **Case Study**

In collaboration with LakePharma, Inc. we determined the antibody sequence for an anti-FLAG M2 antibody. We were able to determine the full-length sequence and fill in missing regions of an Edman sequenced publication (Roosild et al, 2006)

#### Valens sequencing

The purified anti-FLAG M2 antibody was digested with five enzymes, and subjected to LC-MS/MS analysis. Valens recovered the full-length sequences for both the heavy and light chains. We aligned the predicted sequences to the published sequences for the anti-FLAG M2 antibody.

		Heavy Chain		
34 Unknown	Valens	EVQLQQSAAELARPGASVKMSCKASGYXFTXYXIHWVKQRPGQGLEWIGYIXPXXGXXXY		
residues in the published	Published	EVQLQQSGGEL?KPGASVKMSCK?SGYTFT?Y?IHW?KQ?-G?GLEWIGYI?P??G??-Y		
sequence (blue)	Valens	NQNFKDETTLTADPSSSTAYMELNSLTSEDSAVYYCARXXXXGXDYWGQGATLTVSS		
	Published	N??FKGK?TL??DKSSSTAYM????LTSEDSAVY-C?R????G?DYWGQGTTLTVSS		
11 Amino acid				
differences		Light Chain		
(orange)	Valens	DVLMTQIPLSLPVSLGDQASISCRSSQXIVHXNGNTYLEWYLLKPGQSPKLLIYKVXNRF		
	Published	DVLMTQ?PLSLPVSLGDQASISCRSSQ?IVH?NGNTYLEWYLQKPGQSP?LLIYKV?NRF		
Some amino acids				
are replaced by 'X'	Valens	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGXHXPYTFGGGTKLEIR		
for confidentiality	Published	SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQG?H?PYTFGGGTKLEIK		

#### **Expression and validation**

Plasmid DNA encoding the full-length antibody was transfected to CHO cells to produce the HM2 antibody. Purified HM2 antibody was tested for binding to multiple proteins in comparison to commercial M2 antibody, and the results showed that they have similar binding profiles and affinity (Kd).

	Kd against FLAG-tagged protein (Octet)	Kd against His-tagged protein (Octet)	Kd against 3x FLAG-tagged protein (Biacore)
НМ2	26±3 nM	No binding	< 1nM
M2 (ref)	25±4 nM	No binding	< 1nM

#### **Using HM2 for purification**

HM2 antibody was chemically conjugated to sepharose beads. The resulting beads were used to purify from a mixture containing a FLAG-tagged protein. As shown in the graphs to the right, the HM2 beads can specifically and efficiently purify FLAG-tagged proteins with a recovery rate greater than 90%. The target protein binding capacity is 13.5 mg per mL of HM2 resin, which is more than 10x better than commercial M2 resin.

