C-tag affinity tag, from routine protein purification to use in a cGMP production process

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Bioprocessing

INTRODUCTION

Epitope tagging is a technique that employs genetic engineering to fuse a known epitope, called an affinity tag, to either the C or N terminus of a recombinant protein. Although the use of an affinity tag simplifies the purification and detection of proteins, the tag can alter functionality and stability of the expressed protein and negatively impact final production yields. C-tag is a versatile and user-friendly affinity tag, overcoming the current challenges and limitations of tags in protein purification and detection.

C-TAG; A REVOLUTIONARY AFFINITY TAG

C-tag is a 4 amino acid affinity tag: **E-P-E-A** (glutamic acid-proline-glutamic acid-alanine), which can be for purification and detection purposes of proteins. The tag offers high affinity and selectivity when used for purification purposes.

Benefits of C-tag:

- Small **inert** tag – limiting effect on protein functionality
- Highly selective when fused at the C-terminus of a protein
- Limits drawbacks of conventional tags such as lack of selectivity, heavy metal waste or limited reusability
- Enabling high target yield and purity from complex mixtures (compared to His6 tag)

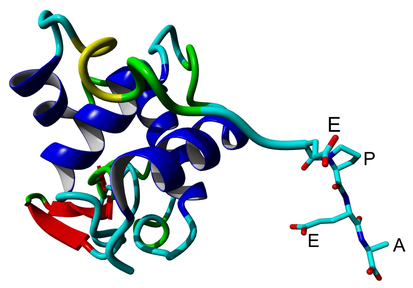


Fig.1 C-tag: The short C-tag sequence (EPEA) fused to a recombinant protein (lysozyme)

CAPTURESELECT AFFINITY PURIFICATION TECHNOLOGY

- Affinity through antibody selectivity: technology based on Camelid single domain [V_HH] antibody fragments
- Unique V_HH screening technology to determine final resin properties such as target specificity, mild elution & ligand stability
- Animal origin free production process (*Saccharomyces Cerevisiae)*
- Technology used in commercial purification processes

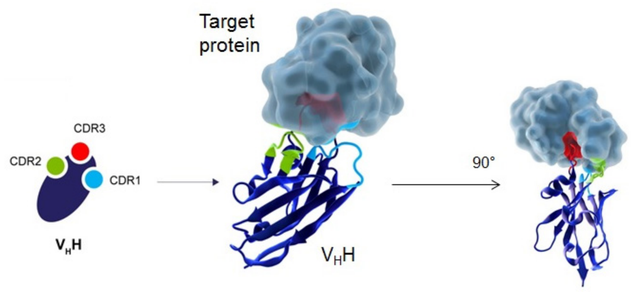


Fig.2 CaptureSelect ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecule.

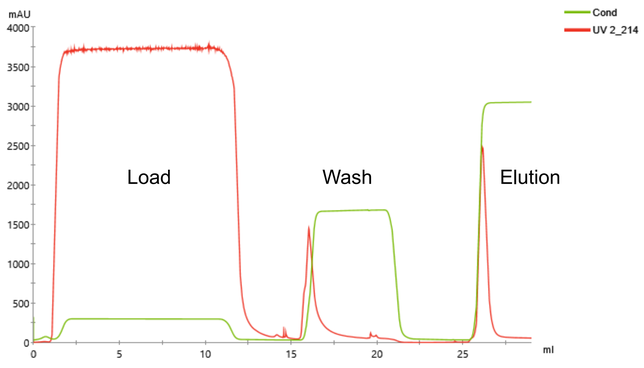
The small size of V_HH fragments (15kD) allows binding at difficult to reach epitopes. Overall, V_HH fragments offer high specificity, affinity and stability.

CAPTURESELECT C-TAG XL AFFINITY MATRIX

Thermo Scientific™ CaptureSelect C-tagXL Affinity Matrix combines a unique selectivity for the small 4-amino acid EPEA tag with the benefits of a robust and high quality affinity matrix. The affinity matrix recognizes the EPEA sequence when fused to linkers between the tag and C-terminus of the protein.

Benefits C-tagXL affinity matrix:

- Enabling high target purity and yield from complex mixtures in a “one-step” process
- Mild elution, protecting the protein of interest
- Scalable



Experimental conditions:
- Eq. buffer: 20mM Tris pH 7.5
- Wash buffer: 20mM Tris pH 7.5 + 1M NaCl
- Elution buffer: 20mM Tris pH 7.5 + 2M $MgCl_2$
- Flow: 150 cm/hour
- Column: 1 ml (0.5cm diameter, 5 cm bed height)

Fig. 3 Chromatogram of the purification of a 13KDa C-tagged protein spiked into a CHO feed. An intermediate wash was introduced to remove nonspecific bound proteins and a pure C-tagged protein was eluted at neutral pH with a MgCl2 elution.

TRADEMARKS/LICENSING

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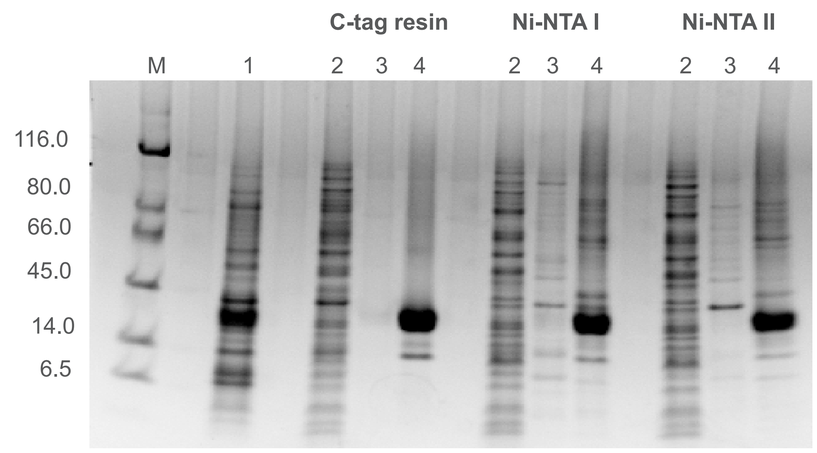
C-TAG APPLICATIONS

In addition to purification of recombinant proteins, C-tag also facilitates detection and quantitation techniques through the use of a biotinylated anti-C-tag ligand (CaptureSelect Biotin Anti-C-tag Conjugate).

Possible applications of C-tag:
- Protein purification, including antibodies and antibody fragments (Fig. 4 & 5)
- ELISA
- Immuno precipitation (IP) and Western Blot
- Label-free detection

Purification examples



M: Mol. weight marker
1: starting material
2: flow through
3: wash
4 elution

Fig 4. Purification of His – and EPEA tagged protein from *E.coli* periplasmatic fraction: C-tag outperforming two different Ni-NTA resins

C-tag is also an ideal platform for the purification of antibodies and antibody fragments such as scFv’s, single VH and VL domains

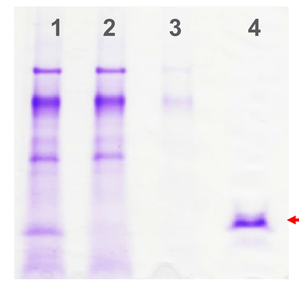


Fig. 5 SDS-PAGE of the purification of a C-tagged V_HH domain
1 Spiked CHO cell culture harvest
2 Flow-through fraction
3 Wash (20 mM Tris, 1M NaCl, 0.05% Tw20 pH 7.5)
4 Elution fraction (20 mM Tris, 2.0 M $MgCl_2$ pH 7.5).

cGMP PURIFICATION OF THE PfRH5 PROTEIN BASED MALARIA VACCINE

For the development and clinical testing of a recombinant protein based malaria vaccine, C-tag was compared to His6 purification. C-tag purification resulted in >85% recovery and >70% purity in a single step. With the use of C-tag, the overall process yield was nearly doubled. C-tag clearly outperformed His6-tag purification (table).

Fig 6A

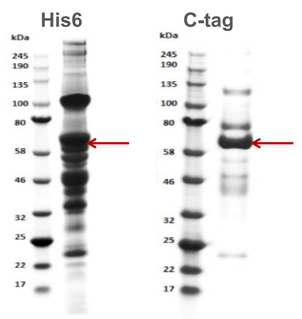
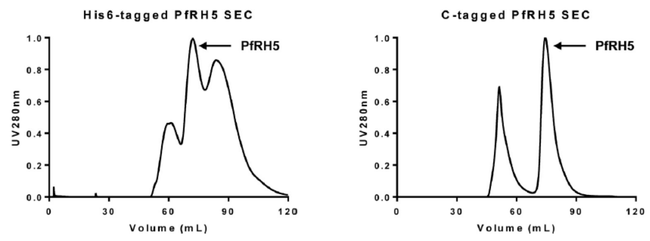


Fig. 6B



Process yield (after)	His6-tagged construct	C-tagged construct
Culture supernatant	100%	100%
Tangential Flow Filtration	82.1%	91.0%
Affinity Chromatography	52.5%	77.4%
Size Exclusion Chromatography	25.5%	43.3%
Overall purity	85-90%	>99%

Fig. 6 Purification of a recombinant malaria vaccine (PfRH5) from insect cells feedstock using a C-terminal fused His6-tag or C-tag.*
A. Purity assessment: Improved purity compared to hexa-histidine tag purification
B. UV280 absorbance chromatograms after Size Exclusion Chromatography (left His6, right C-tag)
C. Process yields after each purification step. C-tag clearly outperforms His6-tag (table)

***Data Obtained from**: Jin, J., et al., 2017. *Accelerating the clinical development of protein-based vaccines for malaria by efficient purification using a four amino acid C-terminal ‘C-tag’*. Int J Parasitol. 47(7), 435-446

CONCLUSION

C-tag has proven to be a versatile affinity tag, useful for the purification and detection of recombinant expressed proteins. CaptureSelect C-tagXL affinity matrix allows for simple and efficient protein production without altering protein functionality, even at cGMP production scale.

Learn more at thermofisher.com/captureselect

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