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

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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.

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 fused at the C-terminus of any recombinant protein. 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
  - Larger tags can alter protein functionality (glutathione S-transferase (GST), Maltose-Binding Protein (MBP), polyhistidine (HIS6))
  - Lack of selectivity for challenging feed stocks (His6)
  - Heavy metal waste using IMAC (His6)
  - Limited reusability / expensive (FLAG™ - Sigma-Aldrich Co.)

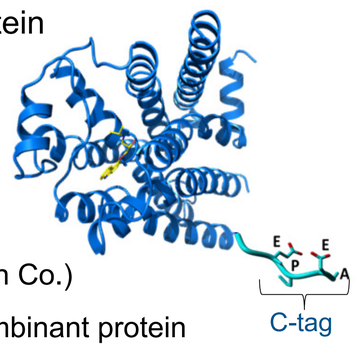

Fig.1 C-tag: The short C-tag sequence (EPEA) fused to a recombinant protein

CaptureSelect™ C-tagXL 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 also recognizes the EPEA sequence when fused to linkers between the tag and C-terminus of the protein.

CaptureSelect Technology – unique affinity purification solution:

- Affinity through antibody selectivity: technology based on Camelid-derived single domain [V_HH] antibody fragments
- Unique screening technology for target specificity, mild elution & stability
- Animal origin free production process (*Saccharomyces cerevisiae)*
- Technology used in commercial purification processes

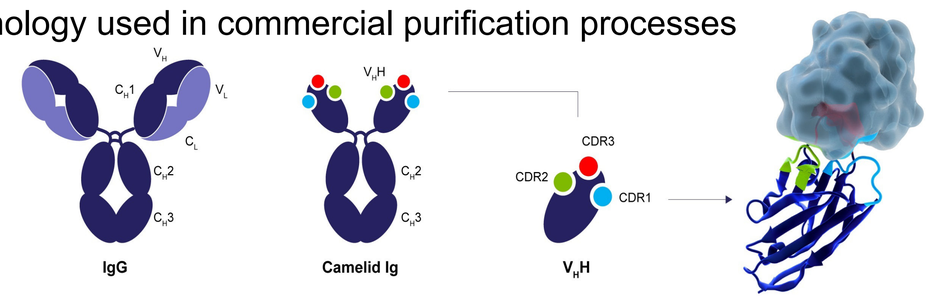

Fig.2 Regular IgG antibody compared to a Camelid heavy-chain antibody. The V_HH antibody fragments offer high specificity, affinity and stability.

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

C-tagXL affinity matrix binding and elution conditions:

Binding Conditions:

Physiological:
- pH range 6 - 8, NaCl up to 150 mM

Denaturing:
- 50 mM Tris, up to 8 M Urea, pH 7
- 50 mM Tris, up to 1 M Guanidine, pH 7

Elution Conditions:

Neutral:
- 20 mM Tris, 2 M $MgCl_2$, pH 7
- 20 mM Tris, 1 M NaCl, 50% (v/v) propylene glycol (PG), pH 7
- 20 mM Tris, 2 mM ”S-E-P-E-A” peptide, ± NaCl, pH 7

Acidic:
- 20 mM citric acid, pH 3
- 100 mM glycine, pH 3

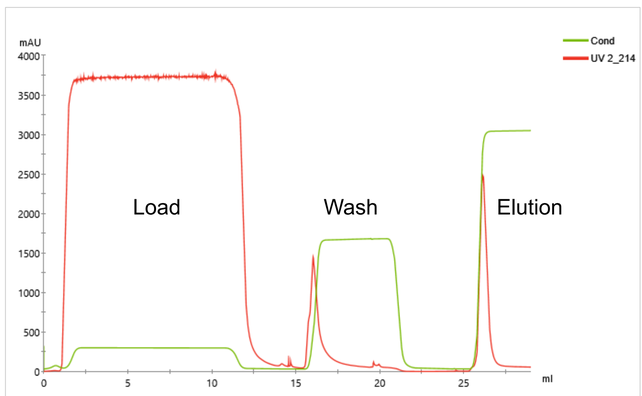

Fig. 3 Chromatogram of the purification of a 13KDa C-tagged protein spiked into a CHO feed. An intermediate wash was introduced to remove nonspecific bound proteins and a pure C-tagged protein was eluted at neutral pH with a MgCl2 elution.

- 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)

C-tag applications

In addition to routine 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 platforms such as Biacore™ and Octet™ (Fig. 6)

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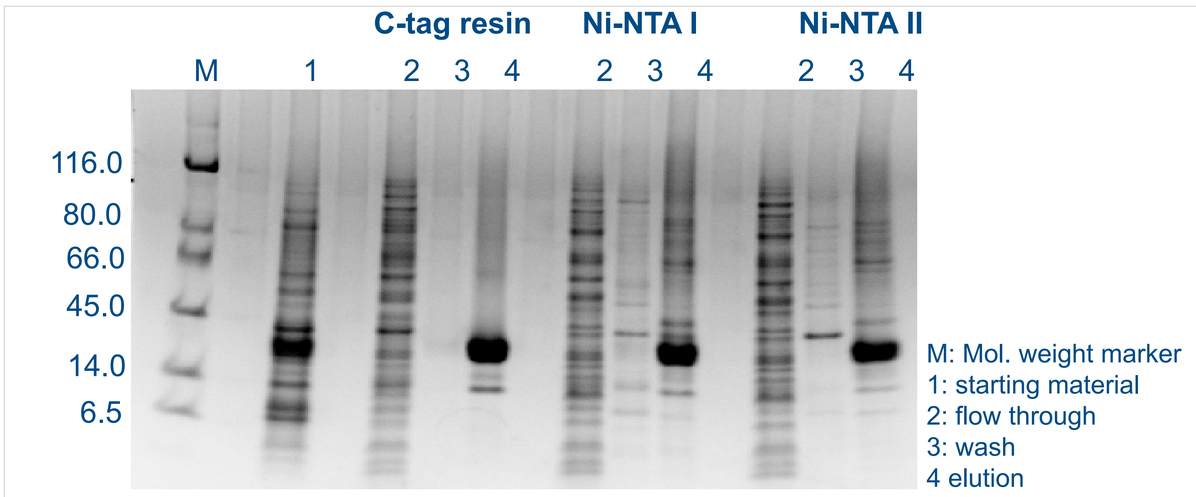

Fig 4. Purification of His –EPEA tagged protein from *E.coli* periplasmatic fraction: C-tag outperforming two different Ni-NTA resins

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

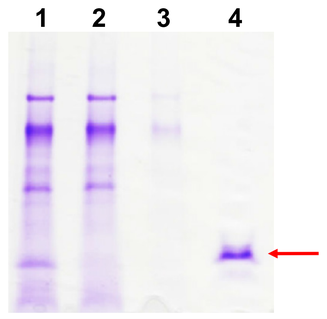

Fig. 5 SDS-PAGE analysis of the purification of a C-tagged V_HH domain
1 Spiked CHO cell culture harvest
2 Flow-through fraction
3 Wash (20 mM Tris, 1M NaCl, 0.05% Tw20 pH 7.5)
4 Elution fraction (20 mM Tris, 2.0 M $MgCl_2$ pH 7.5).

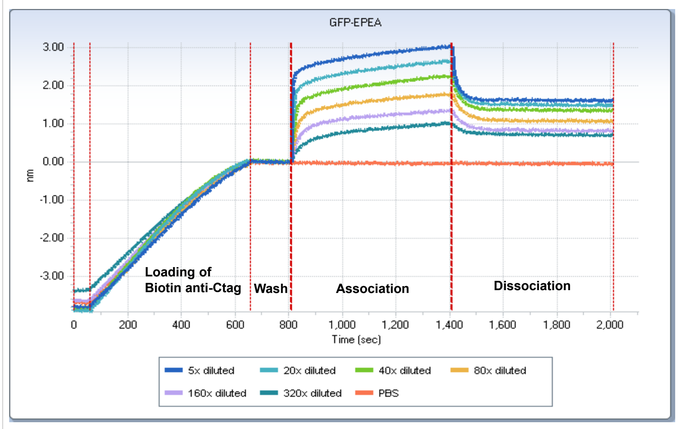

Fig. 6 Label free detection experiment showing the binding analysis of a GFP-EPEA fusion protein. Streptavidin (SA) Biosensors (Octet® QK system) functionalized with Biotin Anti-C-tag Conjugate followed by association and dissociation of crude GFP-EPEA samples at different concentrations.

cGMP purification of a recombinant 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 outporfmed His6-tag purification (table).

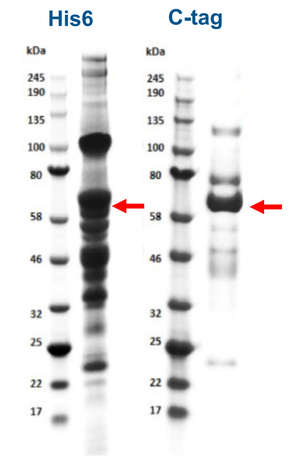

Fig. 7 Purification of a recombinant malaria vaccine (PfRH5) from insect cells feedstock using a C-terminal fused His6 tag or C-tag.

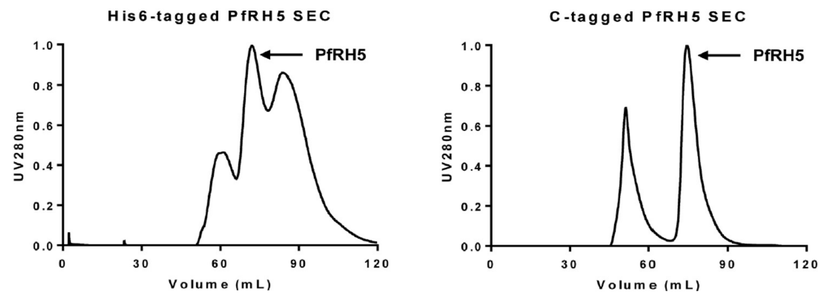

Fig. 8 UV280 absorbance chromatograms after Size Exclusion Chromatography (left His-tagged protein, right C-tagged protein)

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%

Data Fig 7/8 & table obtained from: Jin, J., et al. 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. (2017), http://dx.doi.org/10.1016/j.ijpara.2016.12.001

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 is uniquely designed for the purification of C-tagged proteins, allowing simple and efficient protein production without altering protein functionality, even at cGMP production scale.

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