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), Malto-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.)

**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 inert tag – limiting effect on protein functionality.

**CaptureSelect Technology – unique affinity purification solution:**
- Affinity through antibody selectivity: technology based on Cameldil-derived single domain [V_{H}M] antibody fragments
- Unique screening technology for target specificity, mild elution & stability
- Animal origin free production process (S. cerevisiae)
- Technology used in commercial purification processes

**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.

**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)**

C-tagged protein spiked into a CHO cell culture harvest 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 outperformed His6-tag purification (table).

**Fig. 1**

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

**Fig. 2**

Regular IgG antibody compared to a Cameldil heavy-chain antibody. The V_{H}M antibody fragments offer high specificity, affinity and stability.

**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.

**Fig. 4**

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

**Fig. 5**

SDS-PAGE analysis of the purification of a C-tagged V_{H}M domain

**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.

**Fig. 7**

UV/280 absorbance chromatograms after Size Exclusion Chromatography (left His-tagged protein, right C-tagged protein)

**Fig. 8**

UV/280 absorbance chromatograms after Size Exclusion Chromatography (left His-tagged protein, right C-tagged protein)

<table>
<thead>
<tr>
<th>Culture supernatant</th>
<th>His6-tagged construct</th>
<th>C-tagged construct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity Chromatography</td>
<td>82.1%</td>
<td>77.4%</td>
</tr>
<tr>
<td>Size Exclusion Chromatography</td>
<td>25.5%</td>
<td>43.3%</td>
</tr>
<tr>
<td>Overall purity</td>
<td>88.66%</td>
<td>99.9%</td>
</tr>
</tbody>
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