CaptureSelect™ C-tagXL Affinity Matrix

Catalog Numbers 1943072250, 1943072500, 194307201L, and 194307205L

Pub. No. MAN0017302 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The CaptureSelect™ C-tagXL Affinity Matrix combines unique selectivity for a small 4-amino-acid "*C-tag*" peptide tag **E-P-E-A** (glutamic acid - proline - glutamic acid - alanine) with the benefits of a robust and high-quality affinity matrix provided by a 13 kDa Camelid antibody fragment.

The CaptureSelect™ C-tagXL Affinity Matrix recognizes the E-P-E-A peptide when this tag is fused directly to the C-terminus of a protein (see Figure 1).

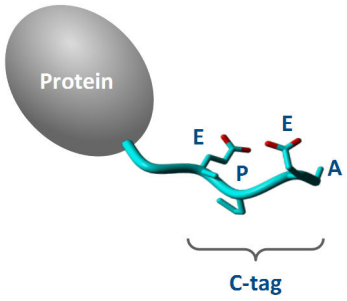

Fig. 1 Representation of a CaptureSelect™ C-tag peptide (E-P-E-A), genetically fused at the C terminus of a recombinant protein.

Note: The Alanine residue (A) of the E-P-E-A sequence needs to remain "free" in order to facilitate proper binding of the CaptureSelect™ C-tagXL Affinity Matrix. You can incorporate linkers between the protein and the C-tagXL as long as the E-P-E-A sequence is displayed at the C-terminal end of the protein of interest.

Product advantages

Epitope tagging is a well-known technology used to facilitate the purification and detection of recombinant proteins for which no suitable ligand is available. The technology allows high-selective capture, but has limitations, especially when you use large tags that can alter protein function, such as GST and MBP. For this reason, the technology is usually used with smaller sequences such as hexa histidine- or FLAG-tag. The hexa histidine-tag, however, often lacks good purity when the protein of interest is extracted from complex mixtures using immobilized metal ion chromatography (IMAC). Peptide tag-antibody systems, such as FLAG-tag, provide a higher selectivity, but are hampered in reusability, due to the poor stability of monoclonal antibodies in a chromatographic set-up, and inefficient binding of target proteins under denaturing conditions such as 8 M urea.

The CaptureSelect™ C-tagXL Affinity Matrix purifies C-terminal tagged proteins with high affinity and selectivity, even in the presence of urea and guanidine HCl, from complex mixtures like cyto - or periplasmic fractions in a one step process. Mild elution conditions at neutral pH can be applied using magnesium chloride or propylene glycol, which ensures high activity recoveries of pH-sensitive target proteins.

The unique features of the CaptureSelect™ C-tagXL system make the system an excellent alternative to sequences such as His - and FLAG-tag, especially for use in a generic purification strategy within a high-throughput protein production environment. The CaptureSelect™ C-tagXL Affinity Matrix offers:

- High recovery and purity in a single step
- Binding under denaturing conditions
- Mild elution to retain the biological activity of C-tagXL fusion proteins
- Compatibility with FPLC systems

Specifications

Ligand	CaptureSelect™ C-tagXL
Binding specificity	C-terminal E-P-E-A
Matrix and particle size	Epoxide-activated agarose, 65 µm
Dynamic binding capacity	400 nmol/mL
Shipping solution	20% (v/v) ethanol

Conditions for use

Parameter	Conditions for use
Equilibration/wash buffer	• Physiological — 20 mM Tris or PBS, pH 7.0–7.4 • Denaturing — – 50 mM Tris, up to 8 M Urea, pH 7.0–7.4 – 50 mM Tris, up to 1 M guanidine, pH 7.0–7.4
Elution buffer	• Neutral (pH 7.0–7.4): – 20 mM Tris, 2.0 M $MgCl_2$ – 20 mM Tris, 1 M NaCl, 50% (v/v) propylene glycol (PG) – 20 mM Tris, 2 mM "S-E-P-E-A" peptide, ±NaCl or $MgCl_2$ • Acidic: – 20 mM citric acid, pH 3 – 100 mM glycine, pH 2–3
Flow rate	100–200 cm/hr
Pressure limit	≤2 bar
pH	2–9
Cleaning solution	Any of the following, prepared freshly every 2–3 days and stored protected from light to minimize radicals that affect the functionality of the matrix: • Citric acid or acetic acid, 0.5–1 M • 6.0 M Urea • PAB (120 mM phosphoric acid, 167 mM acetic acid, and 2.2% (v/v) benzyl alcohol)
Storage solution and temperature	20% (v/v) ethanol • **Short term**: Room temperature • **Long term**: 4°C

About binding under denaturing conditions

The CaptureSelect™ C-tagXL system provides a high level of selectivity, and also allows the protein of interest to be bound under denaturing conditions. After the protein is bound to the matrix (for example, in the presence of 8 M urea), you can incubate the affinity matrix-suitable buffer to allow on-column re-folding before elution.

Note: The stability in Guanidine-HCl is limited to 1 M.

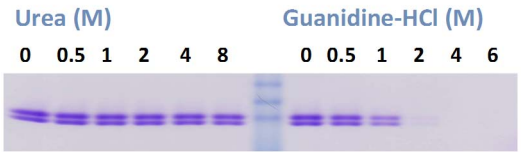

Fig. 2 Elution fractions of a protein tagged with C-tagXL, after binding to the C-tagXL affinity matrix using different concentrations of denaturing agents (urea or guanidine-HCl). After re-folding in PBS, the captured protein was eluted using 100 mM glycine pH 3.

Guidelines for use - FPLC

For optimal matrix performance, optimize the conditions in the guidelines below for your application.

1. Pack the column as described in *CaptureSelect™ Affinity Matrices: Guidelines for Packing* (Pub. No. MAN0009645).
2. Attach the packed column to the FPLC system.
3. Equilibrate with 10 CVs of equilibration/wash buffer.
4. Determine the volume of sample to load, based on the dynamic binding capacity (450 nmol/mL), concentration of the target molecule, and the column size. Optimum loading is at physiological pH. Avoid acidic conditions which decrease binding efficiency.
5. Load the sample on the column.
6. Wash the sample with 5–10 CVs of equilibration/wash buffer. You can increase the NaCl or $MgCl_2$ concentration up to 1.0 M to optimize washing efficiency.
7. Elute with 3–5 CVs of elution buffer.
8. Re-equilibrate the column in equilibration/wash buffer.
9. Strip the column with 0.1 M glycine (pH 2.0), citric acid, or acetic acid (0.5–1.0 M).
10. Re-equilibrate the column in equilibration/wash buffer to prepare the column for another purification run.
11. If the column will not be used immediately, the matrix should be stored in 20% ethanol at 4°C.

Column cleaning guidelines

1. Pump cleaning solution through the column for 15 minutes.
2. Incorporate a static hold to increase the time that cleaning solution is in the column and to minimize the volume of cleaning solution required.

Resin binding capacity

The rate of resin degradation depends on how the resin is used and cleaned. Evaluate individual purification processes. Acidic cleaning procedures should be used for CaptureSelect™ C-tagXL due to the limited stability in buffers above pH 9. The resin is also in Urea up to 6.0 M, but the stability in Guandine HCl is limited to 1.0 M.

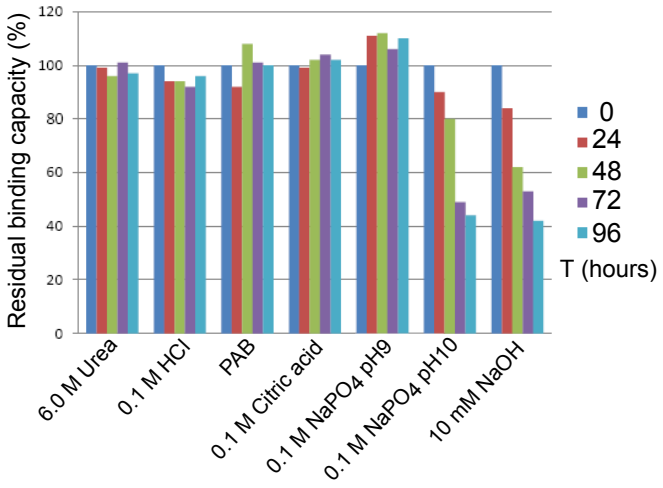

Fig. 3 Residual binding capacity

The CaptureSelect™ C-tagXL Affinity Matrix was exposed to several cleaning agents for up to 96 hours at ambient temperature. The functionality of the resin was measured every 24 hours to test compatibility of the matrix with these cleaning agents.

Example purification of a C-tagged protein

The following examples show the purification of a Camelid domain antibody with a C-terminal E-P-E-A tag that is spiked in CHO cell culture harvest. An intermediate wash was introduced to remove non-specific bound proteins and a pure C-tagged protein was eluted at neutral pH with a $MgCl_2$ elution.

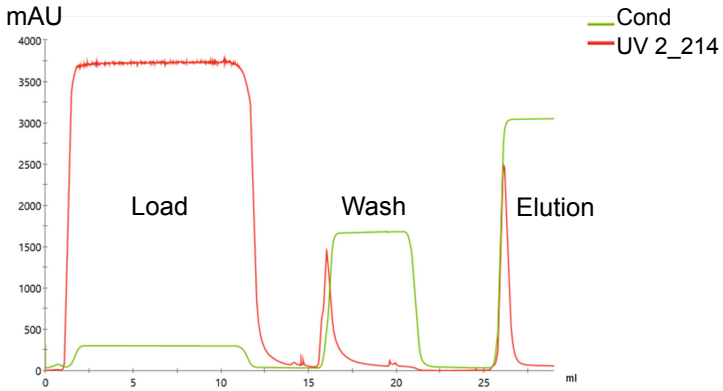

Fig. 4 Chromatagram of the purification of a C-tagged protein

CHO cell culture harvest on a 1-ml column (5x50 mm), flow rate 150 cm/h. Red line: OD214 nm, Green line: Conductivity. Equilibration buffer 20 mM Tris pH 7.5, wash buffer 20 mM Tris, 1.0-M NaCl, 0.05 (w/v)% Tween-20 pH 7.5, elution buffer: 20 mM Tris, 2.0 M $MgCl_2$ pH 7.5.

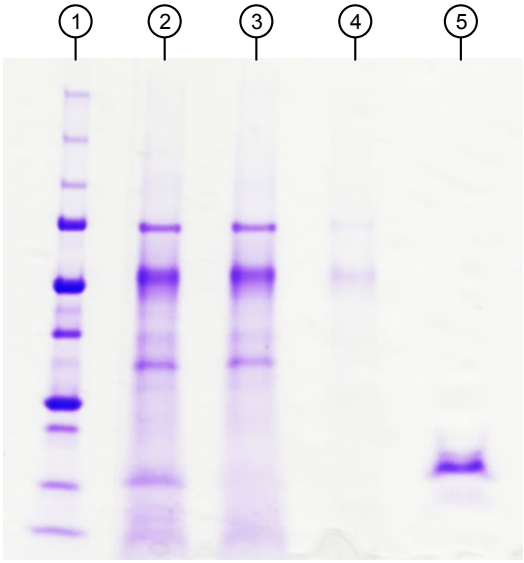

Fig. 5 SDS-PAGE of the purification of a C-tagged protein

CBB stained SDS-PAGE analysis of the purification of a C-tagged antibody fragment that is spiked in CHO cell culture harvest.

① Precision plus marker
② Spiked CHO cell culture harvest
③ Flowthrough fraction
④ Wash fraction
⑤ Elution fraction

C-tagged detection and quantitation

In addition to the CaptureSelect™ C-tagXL Affinity Matrix, the anti-C-tag affinity ligand is also available as a Biotin ligand conjugate (CaptureSelect™ Biotin Anti-C-tag Conjugate) in order to facilitate the easy detection and/or quantitation of C-tagXL fusion proteins in, for example, ELISA, Western blot, and label-free platforms such as those based on surface plasmon resonance (Biacore® and IBIS-MX96 systems) and bio-layer interferometry (ForteBio® Octet® systems). See the *CaptureSelect™ Biotin Anti-C-tag Conjugate Product Information Sheet* (Pub. no. MAN0010067). A ligand leakage ELISA is available for detecting possible leached ligand in the elution fractions of the CaptureSelect C-tagXL affinity matrix.

Ordering Information

Product	Cat. No.
CaptureSelect™ Biotin Anti-C-tag Conjugate	7103252100 (100 µg) 7103252500 (500 µg)
CaptureSelect™ C-tagXL Affinity Matrix	2943072005 (5 mL) 2943071010 (10 mL) 2943072050 (50 mL) 1943072250 (250 mL) 1943072500 (500 mL) 194307201L (1 L) 194307205L (5 L)
CaptureSelect™ C-tagXL Ligand Leakage ELISA	810307201 810307210

For more information

For more information on CaptureSelect™ products and ligand leakage ELISA products, go to **www.thermofisher.com/captureselect**.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

Djender, S. *et al.* 2014. The Biotechnological Applications of Recombinant Single-Domain Antibodies are Optimized by the C-Terminal Fusion to the EPEA Sequence (C Tag). *Antibodies* 3:182–191.

De Genst, E.J. *et al.* 2010. Structure and properties of a complex of α-synuclein and a single-domain camelid antibody. *J Mol Biol.* 402(2): 326–43.

Manufacturer: BAC BV | Huizerstraatweg 28 | 1411 GP Naarden | The Netherlands

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0017302

Revision	Date	Description
A.0	12 September 2017	New document.