CaptureSelect C-tag *affinity matrix*

Epitope tagging is a well known technology to facilitate purification and detection of recombinant proteins for which no suitable ligand is available that allows high selective capture. This technique does have limitations, especially when large tags like GST and MBP are used that can alter protein function. Smaller sequences like hexa histidine or FLAG-tag are preferred. 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 like FLAG-tag do provide a higher selectivity but are hampered in reusability due to poor stability of monoclonal antibodies in a chromatographic set-up and lack efficient binding of target proteins under denaturing conditions.

The "**CaptureSelect C-tag**" affinity matrix combines the unique selectivity for a small 4 amino acid 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-tag 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 cytoplasm or periplasmatic 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 affinity resin recognizes the E-P-E-A tag sequence when fused either directly to the C-terminus of a protein or through linkers between the C-terminus and the E-P-E-A tag (see Figure 1).

- **Incorporation of CaptureSelect C-tag:**

	Linker	C-tag	MW (kDa)
- Protein C-term -	no linker -	**EPEA**	0.44
- Protein C-term -	***GAA*** -	**EPEA**	0.66
- Protein C-term -	***GYQDY*** -	**EPEA**	1.07

The need for a linker is determined by the target protein. Note that other linkers than illustrated above can be applied as long as the "EPEA" sequence is fused at the C-terminal end of the protein of interest. The CaptureSelect C-tag can also find its use within dual-tagging systems for affinity purification of e.g. mammalian protein complexes.

The dynamic binding capacity of the resin is highly dependent on the tagged protein but in the range of 100 nmol/ml resin.

These unique features of the CaptureSelect C-tag system offers an excellent alternative to e.g. His - and FLAG tag, especially for entailing a generic purification strategy within a high-throughput protein production environment.

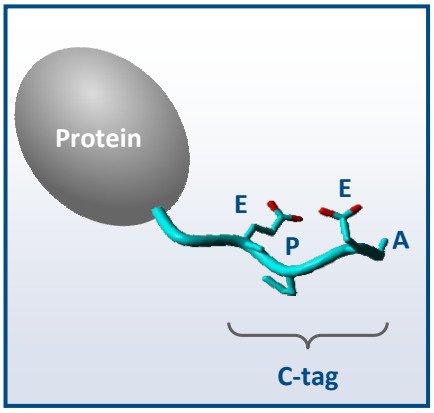


Figure 1 Schematic representation of the CaptureSelect C-tag peptide (E-P-E-A), genetically fused at the C-terminus of a recombinant protein. The Alanine residue at the C-terminal end of the C-tag needs to be "free" in order to facilitate proper binding of the CaptureSelect C-tag affinity ligand.

Product Info

CaptureSelect C-tag	*Affinity Matrix*
Binding capacity	± 100 nmol protein / ml resin
Beads	Low density Glyoxal 6 Rapid Run™ (ABT)*
Average particle size	100 µm
Ligand coupling method	Aldehyde coupling
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
Short term storage:	20% ethanol at room temperature
Long term storage:	20% ethanol at 4 °C, stable for 1 year

*For more information about ABT and the low density glyoxal 6 Rapid Run™ resin please visit:
http://www.abtbeads.com/immobilization_glyoxal.html

Protocols

1 Spin column format *(small scale)*

The CaptureSelect C-tag affinity matrix shows good compatibility with high throughput purification set-ups based on e.g. spin columns or microtiter filter plates. The below protocol describes the use of spin columns and serves as a guideline for similar formats, which can easily be adapted to optimize performance.

- Spin columns (or microtiter plates) can be used with filters having a pore size of less than 100 µm (e.g. MoBiTec cat.no.: M1002S or M105035F). The MoBitec spin columns** can be used for sample volumes up to 700 µl in combination with a 2 ml collection tube.
- Carefully apply 100 - 200 µl of the C-tag affinity matrix to the spin column and wash the resin 3 times by adding a suitable binding buffer (e.g. 500 µl PBS, pH 7.2 - 7.4) and subsequently spin for 1 minute at ≈ 700 g (3000 rpm in eppendorf centrifuge).
- Load the sample to the affinity matrix spin column and incubate for 30 - 60 min (e.g. on a spinning wheel).
- Binding conditions for samples are buffers around pH 6-8 with NaCl up to 150 mM. Ultimately, suitable binding conditions need to be determined empirically. Binding of the target molecule can also be performed in the presence of Urea (up to 8.0 M) and Guanidine HCl (up to 1.0 M).
- Collect flow-through in a 2 ml collection tube: spin for 1 min at 700 g
- Wash the resin 2 - 4 times as described above
- Elute the captured protein of interest by adding 200 - 400 µl elution buffer (see Product Info table) and collect elution fractions by spinning.
- The affinity matrix can be stripped with e.g. 0.1 M Glycine pH 2

** For more information about MoBiTec spin columns please visit: http://www.mobitec.de

2 Packed bed format *(larger scale)*

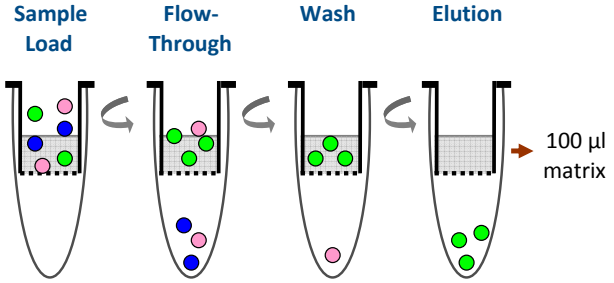
Below a typical chromatography protocol for the CaptureSelect C-tag affinity matrix:

- Carefully pack the CaptureSelect C-tag affinity matrix in a column and equilibrate the matrix by adding a suitable binding buffer (e.g. PBS, pH 7.2-7.4).
- Sample can be applied on the column using an appropriate binding buffer (see Spin column protocol). The amount of sample that can be loaded is depending on the concentration of the target molecule in your sample and the dynamic binding capacity of the matrix. The dynamic binding capacity for C-tagged proteins is about 100 nmol/ml resin, using a linear flow of 150 cm/h.
- After sample application, the column should be washed with binding buffer until baseline has been re-established. A typical wash is 5-10 column volumes (CV).
- Elution of the target molecule from the C-tag affinity matrix is achieved by using 5 CV of a suitable elution buffer (see Product Info table) using a linear flow up to 300 cm/hr.
- The affinity matrix can be stripped with e.g. 0.1 M Glycine pH 2 followed by re-equilibration in binding buffer to allow a second affinity purification run. If the column will not be used immediately, the matrix should be stored in 20% ethanol at 4 °C (39°F).

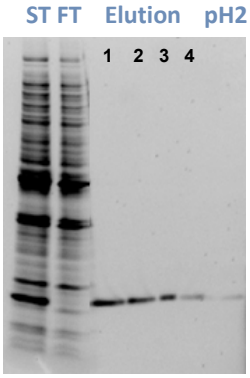
Application Examples

Protein-C-tag Purification *(Spin columns)*

The CaptureSelect C-tag affinity matrix provides excellent selectivity for purification of recombinant proteins equipped with the C-tag from complex mixtures like periplasmatic - or cytoplasmatic fractions from *E.coli* derived expression systems. Besides packed bed columns, the affinity matrix also shows good compatibility with high-throughput purification set-ups. In the below examples, crude samples were applied in filter spin-columns (MoBiTec) each containing 100 µl of the CaptureSelect C-tag affinity matrix (see cartoon below). After sample incubation and washing, the bound C-tagged proteins were eluted using 2 M $MgCl_2$ at neutral pH followed by a column strip at pH 2. Results are displayed in Figures 2 and 3.



A) Protein-***GYQDY***-EPEA



B) Protein-***GAA***-EPEA

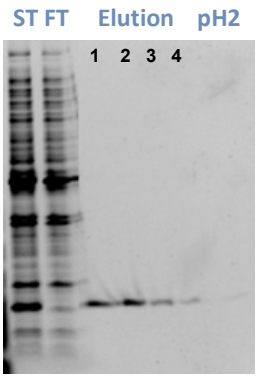


Figure 2 Purification of a Camelid domain antibody equipped with either the C-terminal tag -*GYQDY*-EPEA (A) or -*GAA*-EPEA (B) in a spin column format from *E.coli* derived periplasmatic fractions (400 µl peri / 100 µl affinity matrix per spin column).

Results illustrate very good target recovery and purity from complex mixtures in a one-step process using a neutral pH based elution buffer (20 mM Tris, 2 M $MgCl_2$, pH 7)

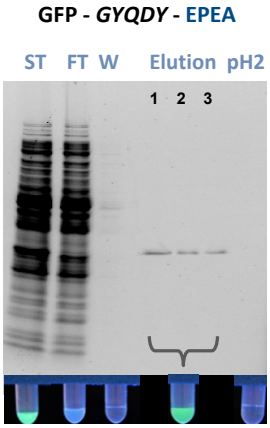


Figure 3 Purification of a green fluorescent protein (GFP) equipped with the C-terminal tag -*GYQDY*-EPEA in a spin column format from an *E.coli* cytoplasmatic fraction (400 µl crude sample / 100 µl affinity matrix per spin column, eluted with 20 mM Tris, 2 M $MgCl_2$, pH7)

Note that for very sensitive proteins that are not compatible with either of the suggested mild elution buffers, release of the captured protein can be obtained by competition using the synthetic peptide "**S-E-P-E-A**" at a concentration of 2 mM in 20 mM Tris, pH 7. More than 60% release is observed using this condition, which can be further optimized by addition of NaCl and/or increasing the S-E-P-E-A peptide concentration.

Binding under denaturing conditions

Besides excellent selectivity provided by the CaptureSelect C-tag system it further allows binding of the protein of interest under denaturing conditions. Once the protein is bound to the matrix in the presence of e.g. 8 M Urea, the affinity matrix can then be incubated with a suitable buffer to allow on-column re-folding prior to elution.

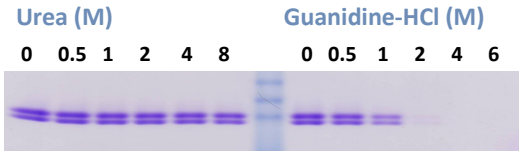


Figure 4 Elution fractions of a C-tagged protein after binding to the C-tag affinity matrix at different levels of denaturing agents (Urea or Guanidine-HCl). After a re-folding step in PBS the captured protein was eluted using 100 mM glycine pH 3.

Dynamic binding capacity *(Packed bed)*

The CaptureSelect C-tag affinity resin can be used in a packed bed format providing good binding capacities at high flow rates (i.e. 150 cm/hr). The below chromatogram illustrates the purification of a Camelid domain antibody equipped with a C-terminal GAA-EPEA tag (MW 14.9 kDa, DBC ≈ 1.7 mg/ml).

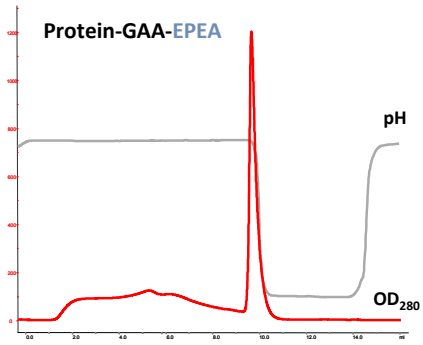


Figure 5 Purification of a Camelid domain antibody equipped with a C-terminal GAA-EPEA tag on a 400 µl Tricorn column (2 cm bed height volume) using an Äkta Explorer system.

Detection and Quantitation of C-tagged proteins

Besides the CaptureSelect C-tag affinity matrix, the anti-C-tag affinity ligand is also provided as conjugated ligand (e.g. conjugated to Biotin, HRPO or AP). This to facilitate easy detection and / or quantitation of C-tagged proteins in e.g. ELISA, western blot and label free platforms like Biacore and Octet (Fortébio).

CaptureSelect C-tag product range

CaptureSelect	Product Code	Size
C-tag affinity matrix	193.3070.05	5 ml affinity matrix
C-tag affinity matrix	193.3070.10	10 ml affinity matrix
C-tag affinity matrix	193.3070.50	50 ml affinity matrix
Biotin anti-C-tag	*710.3070.100*	*in development*
HRPO anti-C-tag	*xxx.3070.xxx*	*in development*
AP anti-C-tag	*xxx.3070.xxx*	*in development*

For more information on CaptureSelect affinity products, please visit our website - www.captureselect.com - or send an E-mail to ligands@captureselect.com.

Warranty

CaptureSelect C-tag affinity matrix and conjugates are supplied for research use only. If you are not satisfied with the performance of BAC BV products, please contact us at ligands@captureselect.com.