CaptureSelect™ Biotin Anti-C-tag Conjugate

Catalog Number 7103252100 and 7103252500

Pub. No. MAN0010067 **Rev.** B.0

Cat. no.	Quantity	Contents	Storage conditions
7103252100	100 µg	1 mg/mL protein in PBS, pH 7.4 (no preservatives added)	• 4°C for short-term storage (up to 1 month) • –5°C to –30°C for long-term storage (aliquot to prevent repeated freeze/thaw cycles)
7103252500	500 µg	1 mg/mL protein in PBS, pH 7.4 (no preservatives added)	

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

CaptureSelect™ Biotin Anti-C-tag Conjugate consists of a 13 kDa Camelid antibody fragment (affinity ligand) with high affinity and selectivity for the 4-amino-acid C-tag peptide **E-P-E-A** (glutamic acid - proline - glutamic acid - alanine).

The Biotin Anti-C-tag affinity ligand recognizes the E-P-E-A peptide when this tag is fused directly to the C-terminus of a protein (see Figure 1).

You can incorporate linkers between the protein and the Biotin Anti-C-tag as long as the E-P-E-A sequence is displayed at the C-terminal end of the protein of interest.

Note: The alanine residue (A) of the E-P-E-A sequence needs to remain free in order to facilitate proper binding of the Biotin Anti-C-tag affinity ligand.

The Biotin Anti-C-tag affinity ligand (constructed as a mono-specific bi-head) is chemically conjugated to biotin via an appropriate spacer that retains the binding reactivity of the ligand when immobilized on streptavidin-functionalized surfaces. The Biotin Anti-C-tag format allows you to:

- **Detect, quantitate, and characterize** – Any C-tag fusion protein when combined with streptavidin- or avidin-based reagents.

Applications for the CaptureSelect™ Biotin Conjugate include Capture ELISA, Western blot, Gyros™ Gyrolab™-based immunoassays, and label-free detection platforms such as those based on surface plasmon resonance (SPR; Biacore™ and IBIS-MX96 systems) and bio-layer interferometry (BLI; ForteBio™ Octet™ systems).

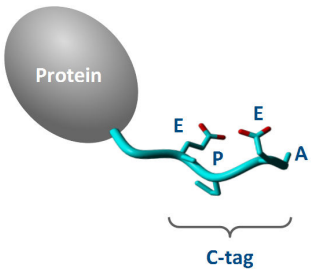

Figure 1 Representation of a CaptureSelect™ C-tag peptide (E-P-E-A), genetically fused at the C terminus of a recombinant protein.

Capture ELISA guidelines for use

Note: Use the recommended materials or their equivalents:

- Buffer – PBS, 0.05% (v/v) Tween™ 20, 1% (w/v) BSA.
- Plates – Nunc MaxiSorp™ flat-bottom 96-well plates. Coat with 1 µg/mL of streptavidin in PBS, 100 µL/well, and let sit overnight at 4°C.
- Detection antibody – Any antibody or protein-binding reagent that has affinity for the protein of interest.

1. Prepare CaptureSelect™ Biotin Conjugate (5 µg/mL in buffer), then add 100 µL/well to the streptavidin-coated plates. Let sit for 1 hour at room temperature to immobilize.
2. Prepare a dilution series of C-tag fusion protein(s). Add 100 µL/well to the Biotin Anti-C-tag-functionalized plates. Let sit for 1 hour at room temperature.
3. Use suitable anti-protein-specific reagents to detect bound protein-E-P-E-A fusion.

Note: If you use Biotin Anti-C-tag Conjugate as a detection antibody, you can combine the conjugate with streptavidin-based reagents, such as those conjugated to enzymes (for example, AP and HRP) or to fluorophores (for example, Alexa Fluor™ and DyLight 488 fluorescent dyes).

Western blot guidelines for use

Note: Use the recommended materials or their equivalents:

- Buffer – PBS, 1% (w/v) skimmed milk, 0.05% (v/v) Tween™ 20.

1. Run the protein sample(s) of interest by SDS PAGE, then transfer the separated proteins onto an appropriate membrane (for example, by electroblotting).
2. Block the membrane for 1 hour at room temperature with 2% (w/v) skimmed milk in PBS.
3. Incubate the blocked membrane with Biotin Anti-C-tag, 1 µg/mL in buffer.
4. Detect bound Biotin Anti-C-tag using streptavidin-AP conjugate, 1:2000 in buffer.
5. Use BCIP/NBT-based substrates (or equivalent substrates suitable for AP) to generate a color reaction.

Western blot application example

In combination with commercially available streptavidin-AP conjugates, the CaptureSelect™ Biotin Conjugate can be used in Western blot for the specific detection and quantitation of C-tag fusion proteins. See Figure 2.

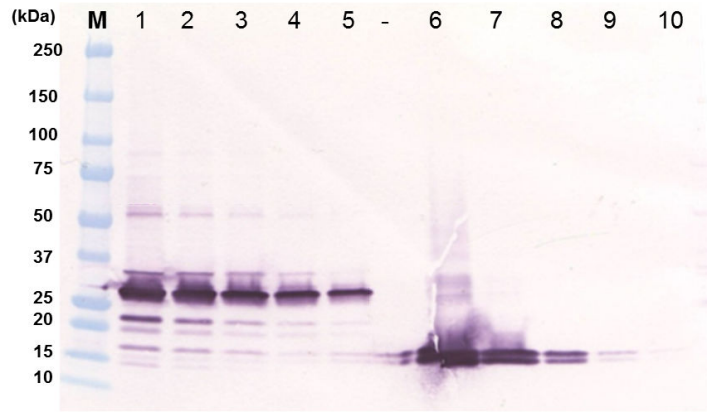

Figure 2 Western blot analysis of GFP (crude lysate) and a single domain antibody (purified), both equipped with the C-tag peptide.

Pre-stained markers:

1. GFP-E-P-E-A fusion, crude *E.coli* lysate, 20X dilution
2. 40X dilution
3. 80X dilution
4. 160X dilution
5. 320X dilution
6. Single domain antibody-E-P-E-A fusion, 400 ng/mL
7. 80 ng/mL
8. 15 ng/mL
9. 3.0 ng/mL
10. 0.6 ng/mL

Label-free and real-time binding assays

The CaptureSelect™ Biotin Conjugate can be used in label-free and real-time binding assays such as bio-layer interferometry (BLI) and surface plasmon resonance (SPR). Both systems provide streptavidin-linked biosensors that can immobilize biotinylated affinity ligands for use as capturing agents to measure interactions with any protein that has been equipped with the C-tag peptide.

Bio-layer interferometry (BLI) guidelines for use

Note: Use the recommended materials or their equivalents.

1. Load prepared CaptureSelect™ Biotin Conjugate (5 µg/mL in 200 µL of PBS) on ForteBio™ Streptavidin (SA) Biosensors for 10 minutes at a shake speed of 400 rpm, then wash with PBS for 2.5 minutes.
2. Bind the C-tag fusion protein (in PBS) for 10 minutes at a shake speed of 1000 rpm, then dissociate with PBS for 10 minutes.
3. *(Optional)* Regenerate the biosensors with 0.1 M glycine, pH 2, for 5 minutes at a shake speed of 1000 rpm.

BLI application example

The CaptureSelect™ Biotin Conjugate is highly compatible with ForteBio™ Streptavidin (SA) Biosensors, and can be used in a range of applications for protein analytics on the Octet™ platform. See Figure 3.

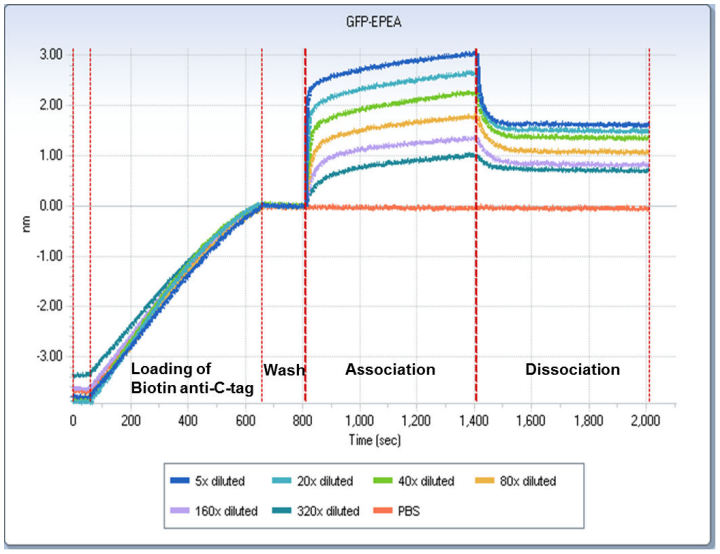

Figure 3 Binding analysis of GFP-E-P-E-A fusion protein demonstrates ForteBio™ Streptavidin (SA) Biosensors (Octet™ QK system) functionalized with Biotin Anti-C-tag Conjugate followed by association and dissociation of crude GFP-E-P-E-A samples at different concentrations.

Surface plasmon resonance (SPR) guidelines for use

Note: Use the recommended materials or their equivalents.

1. Load prepared CaptureSelect™ Biotin Conjugate (10 µg/mL in HBS-EP buffer) onto a Biacore™ Sensor Chip SA (Biacore™ 3000 system) at a flow rate of 10 µL/minute for at least 3 minutes.
2. Bind the C-tag fusion protein (in HBS-EP buffer) at a flow rate of 5 µL/minute for 1 minute.
3. Dissociate in HBS-EP buffer at a flow rate of 5 µL/minute for 2.5 minutes.
4. Regenerate after each cycle with 0.1 M glycine, pH 2, at a flow rate of 30 µL/minute for 1.5 minutes.

Protein C-tag purification

In addition to the CaptureSelect™ Biotin Anti-C-tag Conjugate, the anti-C-tag affinity ligand is also available as an affinity matrix (CaptureSelect™ C-tag Affinity Matrix) to facilitate highly selective one-step purification of C-tag fusion proteins from complex mixtures such as cyto or periplasmic fractions from *E.coli*-derived expression systems. Refer to the *CaptureSelect™ C-tag Affinity Matrix Product Information Sheet* (Pub. no. MAN0010706).

Ordering Information

Product	Cat. no.
CaptureSelect™ Biotin Anti-C-tag Conjugate	7103252100 (100 µg) 7103252500 (500 µg)
CaptureSelect™ C-tag Affinity Matrix	191307005 (5 mL) 191307010 (10 mL) 191307050 (50 mL)

For more information

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

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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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.

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