CaptureSelect™ Biotin Anti-LC-lambda (Hu) Conjugate

Catalog Number 7103082100 and 7103082500

Pub. No. MAN0010066 **Rev.** B.0

Cat. no.	Quantity	Contents	Storage conditions
7103082100	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)
7103082500	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-LC-lambda (Hu) Conjugate consists of a 13 kDa Llama antibody fragment (affinity ligand) that specifically binds to the constant domain of human lambda light chains (see Figure 1).

The affinity ligand 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-LC-lambda (Hu) format allows you to:

- **Detect, quantitate, and characterize** — All human antibody isotypes and subclasses (for example, IgG1 to 4, IgM, IgA, IgD, and IgE), and Fab fragments thereof, that contain a lambda light chain; as well as recombinant human Fab fragments and/or Fab and Fab2 fragments obtained after papain or pepsin digestion.
- **Avoid cross-binding** — Biotin Anti-LC-lambda (Hu) does not cross-bind with human antibodies that possess a kappa light chain or IgG from, for example, mouse, rat, or rabbit.
- **Screen antibody-antigen interactions** — Biotin Anti-LC-lambda (Hu) forms a stable [low k(diss)] complex with, for example, human IgG antibodies through bivalent binding.

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).
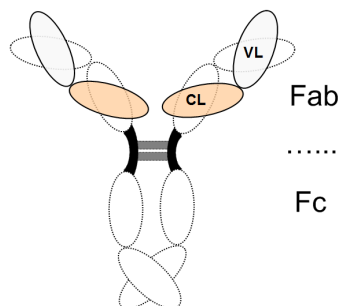

Figure 1   Representation of an IgG antibody. The constant domain of the human lambda light chain (in orange) is recognized by Biotin Anti-LC-lambda (Hu).

Binding selectivity: Biotin Anti-LC-lambda (Hu)

Antibody target	Isotype/subclass	Binding selectivity [1]
IgG subclasses	Human IgG1	✓
	Human IgG2	✓
	Human IgG3	✓
	Human IgG4	✓
Ab isotypes	Human IgM	✓
	Human IgD	✓
	Human IgE	✓
	Human IgA	✓
Ab fragments	Human IgG Fab	✓
	Human IgG Fc	–
	Free human LC kappa	–
	Free human LC lambda	✓
Ab species	Mouse, rat, rabbit	–
	Sheep	–
	Bovine, goat	✓
	Chimpanzee, rhesus, cynomolgus	✓

[1] Mono- or polyclonal antibodies containing a light chain of the human lambda isotype. No binding observed for kappa light chain antibodies.

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 – Goat anti-human-IgG (H+L) or anti-human-IgG (Fc) HRP conjugates, such as those from Bio-Rad™ Laboratories.

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 antibody samples that contain human lambda light chains. Add 100 µL/well to the Biotin Anti-LC-lambda (Hu)-functionalized plates. Let sit for 1 hour at room temperature.

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3. Use commercially available detection antibodies to detect bound antibody molecules.
4. Use TMB/H$_2$O$_2$-based substrates (or equivalent substrates suitable for HRP) to generate a color reaction.

Note: To achieve good assay sensitivity or LLOD (lower limit of detection), you must optimize the ELISA conditions. We recommend using antibody-specific conjugates for detection to limit serum-induced background signals. Background signals may vary between serum samples.

Capture ELISA application example

When immobilized on streptavidin-coated microtiter plates, Biotin Anti-LC-lambda (Hu) can be used as a capturing agent in highly sensitive assays to detect and quantitate human antibodies and Fab fragments that contain a lambda light chain. You can detect captured antibody molecules using commercially available secondary antibody reagents. See Figure 2.
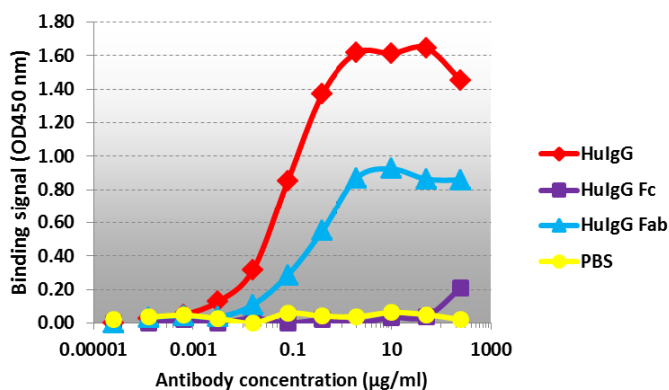

Figure 2 Example dose-response curves for polyclonal human IgG, IgG Fc, and IgG Fab in Capture ELISA using Biotin Anti-LC-lambda (Hu) as the capturing agent. The polyclonal samples have a purity of approximately 97%.

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 under non-reducing conditions, then transfer the separated proteins onto an appropriate membrane (for example, by electroblotting).

   Note: When protein samples are run under reducing conditions, we have observed poor to no binding with Biotin Anti-LC-lambda (Hu) in Western blot applications.

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-LC-lambda (Hu), 1 µg/mL in buffer.
4. Detect bound Biotin Anti-LC-lambda (Hu) 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 of human antibodies and Fab fragments that contain a lambda light chain. See Figure 3.

M Precision Plus Protein™ All Blue Standards marker (Bio-Rad®)
1. Polyclonal HuIgG
2. Polyclonal HuIgG Fc
3. Polyclonal HuIgG Fab
4. Polyclonal HuIgG LC lambda
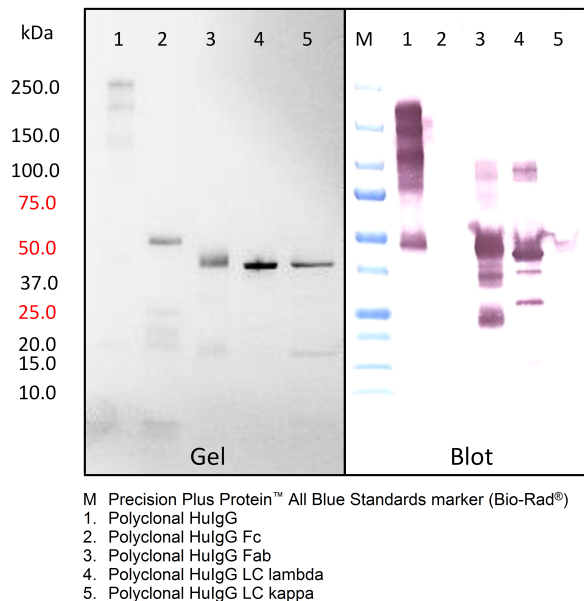5. Polyclonal HuIgG LC kappa

Figure 3 Western blot analysis of different polyclonal human IgG antibodies (non-reduced) using Biotin Anti-LC-lambda (Hu). The polyclonal samples have a purity of approximately 97%.

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 human antibody format that contains a human lambda light chain.

Note: When CaptureSelect™ Biotin Anti-LC-lambda (Hu) Conjugate is used in combination with CaptureSelect™ Biotin Anti-LC-kappa (Hu) Conjugate (Cat. nos. 7103272100 and 7103272500), any human immunoglobulin and/or antibody format that contains a light chain of the human species is covered.

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 antibody target samples (0.1–250 µg/mL 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.

CaptureSelect™ Biotin Anti-LC-lambda (Hu) Conjugate Product Information Sheet

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 antibody analytics on the Octet™ platform. See Figure 4 and Figure 5.
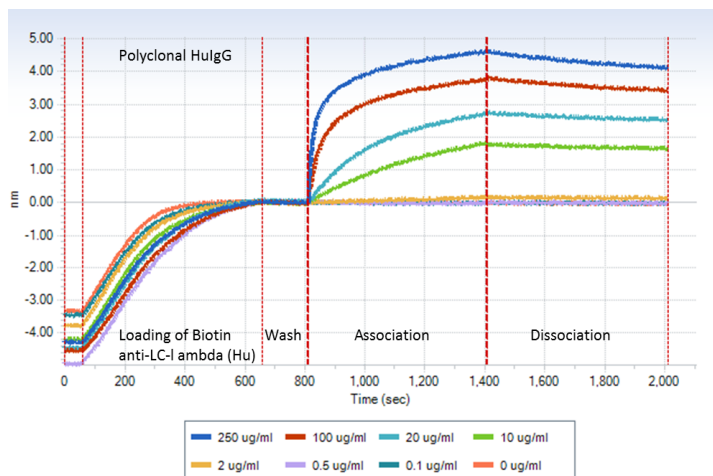

Figure 4   Binding analysis of polyclonal human IgG demonstrates ForteBio™ Streptavidin (SA) Biosensors (Octet™ QK system) functionalized with Biotin Anti-LC-lambda (Hu) followed by association and dissociation of polyclonal human IgG samples at different antibody concentrations.
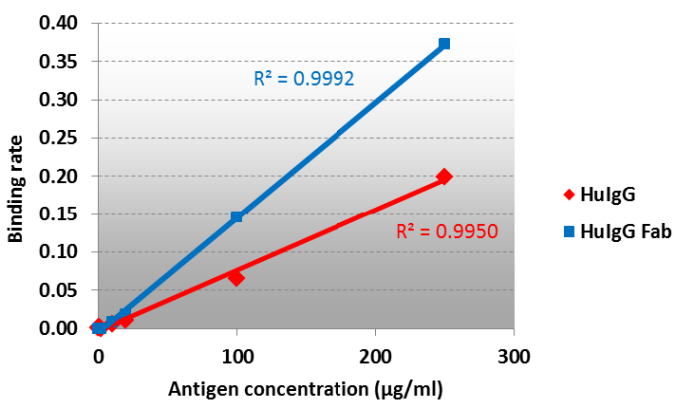
Figure 5   Example calibration curves of polyclonal human IgG and IgG Fab on Biotin Anti-LC-lambda (Hu)-functionalized biosensors. To demonstrate the use of Biotin Anti-LC-lambda (Hu) for quantitation purposes, binding rates were obtained for the first 10 seconds of association.

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 antibody target samples (10 µg/mL 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.

SPR application example

The CaptureSelect™ Biotin Conjugate is compatible with the Biacore™ Sensor Chip SA and the Biacore™ Biotin CAPture Kit, which enables reversible capture of biotinylated molecules and standardized regeneration for interaction studies. See Figure 6 and Table 1.
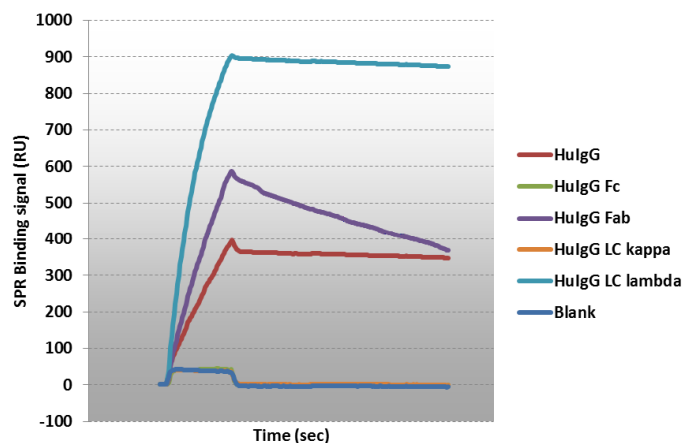

Figure 6   Association and dissociation curves of polyclonal human IgG antibodies (IgG, IgG Fc, IgG Fab, IgG LC kappa, and IgG LC lambda) on Biacore™ Sensor Chips SA (Biacore™ 3000 system) functionalized with Biotin Anti-LC-lambda (Hu).

Table 1   Relative binding selectivity for human antibodies and Fab fragments that contain a lambda light chain on a Biotin Anti-LC-lambda (Hu)-functionalized Biacore™ Sensor Chip SA (Biacore™ 3000 system).

IgG subclasses (monoclonal)	Binding[1]	IgG fragments/isotypes (polyclonal)	Binding[1]
Human IgG1 kappa	-	Human IgG Fc	-
Human IgG1 lambda	++	Human IgG Fab	++
Human IgG2 kappa	-	Free human LC kappa	-
Human IgG2 lambda	++	Free human LC lambda	++
Human IgG3 kappa	-	Human IgM	+
Human IgG3 lambda	++	Human IgA	+
Human IgG4 kappa	-	Human IgD	+
Human IgG4 lambda	++	Human IgE	+

[1] -: <10 RU, +: 10–200 RU, ++: >200 RU

Antibody-antigen screening

To screen monoclonal antibodies (mAbs) on antigen-binding kinetics, it is important to have high-affinity capturing biosensors. Because binding of human IgG antibodies on Biotin Anti-LC-lambda (Hu)-functionalized surfaces is bivalent (binding to both LC-lambda domains on one IgG molecule), dissociation rates are very low (in the range of 10^{-6} s^{-1}, as determined on the Octet™ platform). Low k(diss) values provide stable complexes with mAbs of interest to enable proper antigen-binding kinetics.

Ordering Information

CaptureSelect™ Biotin Conjugates	Cat. no.
Anti-Free LC-kappa (Human)	7103292100 (100 µg)
	7103292500 (500 µg)
Anti-IgA	7102882100 (100 µg)
	7102882500 (500 µg)
Anti-IgG3 (Human)	7103042100 (100 µg)
	7103042500 (500 µg)
Anti-IgG4 (Human)	7102902100 (100 µg)
	7102902500 (500 µg)
Anti-IgG-CH1	7103202100 (100 µg)
	7103202500 (500 µg)
Anti-IgG-Fc (Human)	7103262100 (100 µg)
	7103262500 (500 µg)
Anti-IgG-Fc (Multi-species)	7102852100 (100 µg)
	7102852500 (500 µg)
Anti-IgM	7102892100 (100 µg)
	7102892500 (500 µg)
Anti-LC-kappa (Human)	7103272100 (100 µg)
	7103272500 (500 µg)
Anti-LC-kappa (Murine)	7103152100 (100 µg)
	7103152500 (500 µg)
Anti-LC-lambda (Human)	7103082100 (100 µg)
	7103082500 (500 µg)
Human Fab-kappa Kinetics	7103302100 (100 µg)
	7103302500 (500 µg)
Human Fab-lambda Kinetics	7103312100 (100 µg)
	7103312500 (500 µg)

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
- 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

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