

# CaptureSelect™ Biotin Anti-IgG-CH1 Conjugate

Catalog Number 7103202100 and 7103202500

Pub. No. MAN0010060 Rev. B.0

Cat. no.	Quantity	Contents	Storage conditions
7103202100	100 µg	1 mg/mL protein in PBS, pH 7.4 (no preservatives added)	<ul style="list-style-type: none"> <li>4°C for short-term storage (up to 1 month)</li> <li>-5°C to -30°C for long-term storage (aliquot to prevent repeated freeze/thaw cycles)</li> </ul>
7103202500	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](http://thermofisher.com/support).

## Product description

CaptureSelect™ Biotin Anti-IgG-CH1 Conjugate consists of a 14 kDa Lama antibody fragment (affinity ligand) that specifically binds to the CH1 domain of all four human IgG subclasses (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-IgG-CH1 format allows you to:

- **Detect, quantitate, and characterize** – All human IgG antibodies (subclasses 1 to 4) and Fab fragments thereof, independent of the type of light chain; and total IgG in human plasma and/or serum samples.
- **Avoid cross-binding** – Biotin Anti-IgG-CH1 does not cross-bind with other isotypes such as IgA, IgM, IgD, and IgE, free light chains, or with IgG from mouse sources or bovine sources such as FCS.

**Note:** There is cross-reactivity towards IgG antibodies from certain species; see “Binding selectivity: Biotin Anti-IgG-CH1”.

- **Screen antibody-antigen interactions** – Biotin Anti-IgG-CH1 forms a stable [low k(diss)] complex with 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).

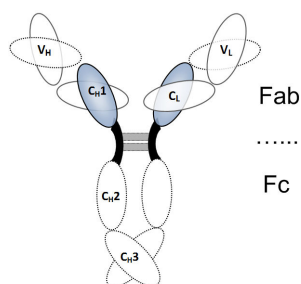


Figure 1 Representation of an IgG antibody. The CH1 domain within the Fab part of human IgG antibodies (in dark blue) is recognized by Biotin Anti-IgG-CH1.

## Binding selectivity: Biotin Anti-IgG-CH1

Antibody target	Isotype/subclass	Binding selectivity [1]
IgG subclasses	Human IgG1	✓
	Human IgG2	✓
	Human IgG3	✓
	Human IgG4	✓
Ab isotypes	Human IgM	-
	Human IgA	-
	Human IgD	-
	Human IgE	-
Ab fragments	Human IgG Fab	✓
	Human IgG Fc	-
	Free human LC kappa	-
	Free human LC lambda	-
Ab species	Bovine	-
	Rat, mouse, rabbit	-
	Swine, goat, sheep	-
	Guinea pig, Syrian hamster, horse	✓
	Chimpanzee, rhesus, cynomolgus	✓
	Cat, dog, donkey	✓

[1] Selectivity analyzed with mono- and/or polyclonal 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 – Mouse anti-human IgG-Fc-HRPO conjugate (such as those from Jackson ImmunoResearch) or any other suitable antigen-specific antibody or compound.

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 samples that contain human IgG or human IgG Fab fragments. Add 100  $\mu\text{L}$ /well to the Biotin Anti-IgG-CH1-functionalized plates. Let sit for 1 hour at room temperature.
3. Use commercially available detection antibodies to detect bound antibody molecules.
4. Use TMB/ $\text{H}_2\text{O}_2$ -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-IgG-CH1 can be used as a capturing agent in highly sensitive assays to detect and quantitate human IgG antibodies (subclasses 1 to 4) and human IgG-derived Fab fragments, without cross-binding with free light chains or IgG from bovine sources such as FCS. You can detect captured antibody molecules using commercially available secondary antibody reagents. See Figure 2.

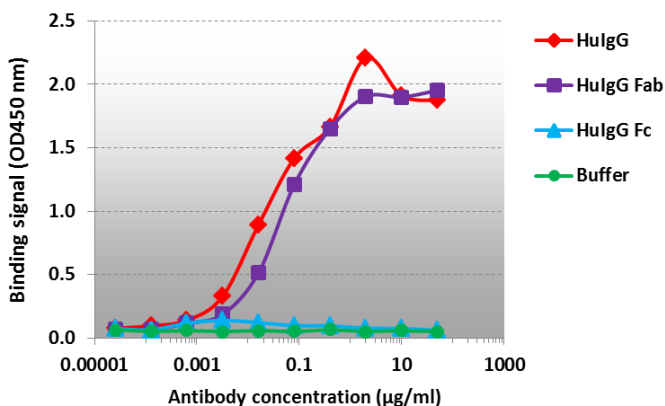


Figure 2 Example dose-response curves for polyclonal human IgG and Fab/Fc fragments in Capture ELISA using Biotin Anti-IgG-CH1 as the capturing agent. The polyclonal samples have a purity of approximately 97%.

### 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-IgG-CH1 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-IgG-CH1, 1  $\mu\text{g}/\text{mL}$  in buffer.
  4. Detect bound Biotin Anti-IgG-CH1 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 IgG antibodies (subclasses 1 to 4) and human IgG-derived Fab fragments, without cross-binding with other isotypes such as IgA, IgD, IgE, and IgM, or IgG from bovine sources such as FCS. See Figure 3.

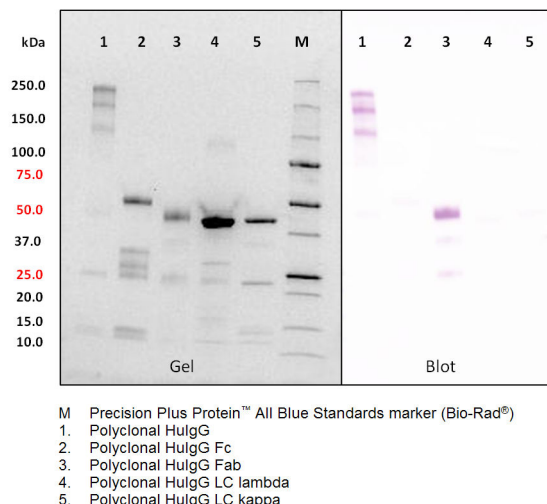


Figure 3 Western blot analysis of different polyclonal human IgG antibodies and fragments (non-reduced) using Biotin Anti-IgG-CH1. The polyclonal samples have a purity of approximately 97%.

### 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 human IgG antibodies or Fab fragments. Because the anti-IgG-CH1 affinity ligand binds to the CH1 domain within the Fab part of an IgG antibody, it provides more consistency in analyzing diverse panels of human IgG Fab fragments than, for example, Protein L. (Protein L shows restricted binding to human VL-kappa 1, 3, and 4 only and lacks binding reactivity towards lambda light chains.)

### Bio-layer interferometry (BLI) guidelines for use

**Note:** Use the recommended materials or their equivalents.

1. Load prepared CaptureSelect™ Biotin Conjugate (5  $\mu\text{g}/\text{mL}$  in 200  $\mu\text{L}$  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 (0.08–50  $\mu\text{g}/\text{mL}$  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.

## 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.

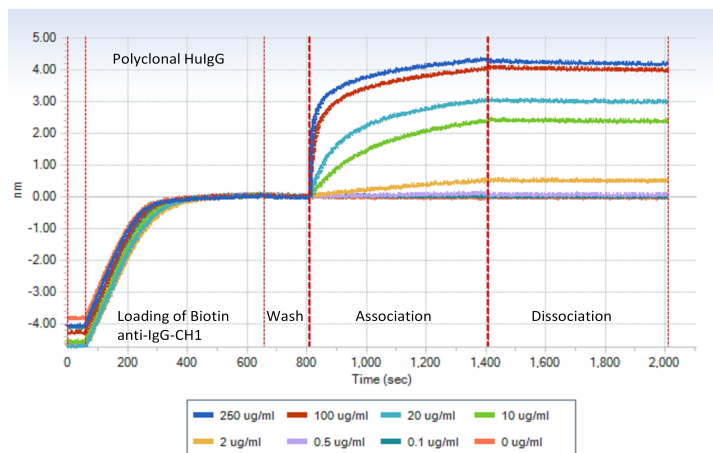


Figure 4 Binding analysis of human IgG demonstrates ForteBio™ Streptavidin (SA) Biosensors (Octet™ QK system) functionalized with Biotin Anti-IgG-CH1 followed by association and dissociation of human IgG samples at different antibody concentrations.

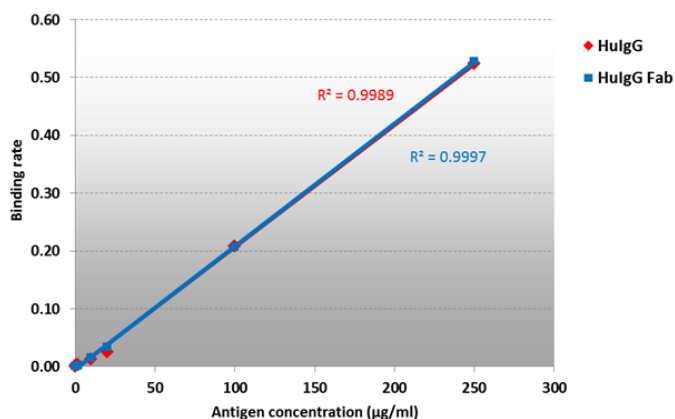


Figure 5 Example calibration curves of human IgG and IgG Fab fragments on Biotin Anti-IgG-CH1-functionalized biosensors. To demonstrate the use of Biotin Anti-IgG-CH1 for quantitation purposes, binding rates were obtained for the first 10 seconds of association.

## 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.

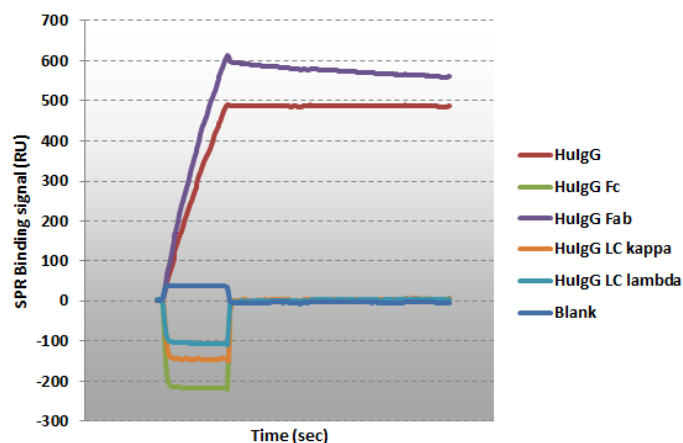


Figure 6 Association and dissociation curves of polyclonal human IgG and Fab/Fc fragments (human IgG, human IgG Fc, human IgG Fab, human IgG LC kappa, and human IgG LC lambda) on Biacore™ Sensor Chips SA (Biacore™ 3000 system) functionalized with Biotin Anti-IgG-CH1.

**Table 1** Relative binding selectivity for human IgG antibodies and Fab fragments thereof on a Biotin Anti-IgG-CH1-functionalized Biacore™ Sensor Chip SA (Biacore™ 3000 system). The Biotin Anti-IgG-CH1 format prevents cross-binding with other isotypes, such as IgA, IgM, IgD, and IgE, or with IgG from mouse sources or bovine sources such as FCS.

IgG subclasses (monoclonal)	Binding <sup>[1]</sup>	IgG fragments/isotypes (polyclonal)	Binding <sup>[1]</sup>
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	-

<sup>[1]</sup> --: <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-IgG-CH1-functionalized surfaces is bivalent (binding to both CH1 domains on one IgG molecule), dissociation rates are very low (in the range of  $10^{-5} \text{ s}^{-1}$ , as determined on the Octet™ platform). Such low  $k(\text{diss})$  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](http://www.thermofisher.com/captureselect).

## Customer and technical support

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  - 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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