CaptureSelect™ Biotin Anti-IgG3 (Hu) Conjugate

Catalog Number 7103042100 and 7103042500

Pub. No. MAN0014342 **Rev.** A.0

Cat. no.	Quantity	Contents	Storage conditions
7103042100	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)
7103042500	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-IgG3 (Hu) Conjugate consists of a 13 kDa Llama antibody fragment (affinity ligand) that specifically binds to human IgG3 subclasses, through recognition of an epitope within the IgG3 hinge region (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-IgG3 (Hu) format allows you to:

- **Detect, quantitate, and characterize** — All human IgG3 subclass antibodies.
- **Avoid cross-binding** — Biotin Anti-IgG3 (Hu) does not cross-bind with the other human IgG subclasses (IgG1, IgG2, and IgG4) or isotypes such as human IgA, IgD, IgE, and IgM, or IgG from bovine sources such as FCS.

Applications for CaptureSelect™ Biotin Anti-IgG3 (Hu) 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).

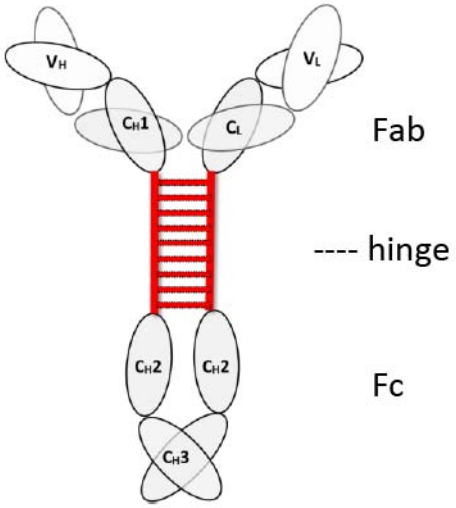


Figure 1 Representation of a human IgG3 antibody.

Binding selectivity Biotin Anti-IgG3 (Hu)

Antibody target	Subclass/Isotype	Binding selectivity [1]
IgG subclasses	Human IgG1	–
	Human IgG2	–
	Human IgG3	✓
	Human IgG4	–
Ab isotypes	Human IgM	–
	Human IgA	–
	Human IgD	–
	Human IgE	–

[1] Cross-binding can be expected on IgGs from non-human primates like Chimpanzee, Cynomologus macaque, and Rhesus macaque.

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 – Mono– or polyclonal anti-human IgG HRP conjugates such as those from Thermo Fisher Scientific

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 IgG3 antibodies Add 100 µL/well to the Biotin Anti-IgG3 (Hu)-functionalized plates. Let sit for 1 hour at room temperature.
3. Use commercially available detection antibodies to detect bound antibody molecules.
4. Use TMB/H_20_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-IgG3 (Hu) can be used as a capturing agent in sensitive assays to detect and quantitate human IgG3 antibodies, without cross-binding with other IgG subclasses. See Figure 2.

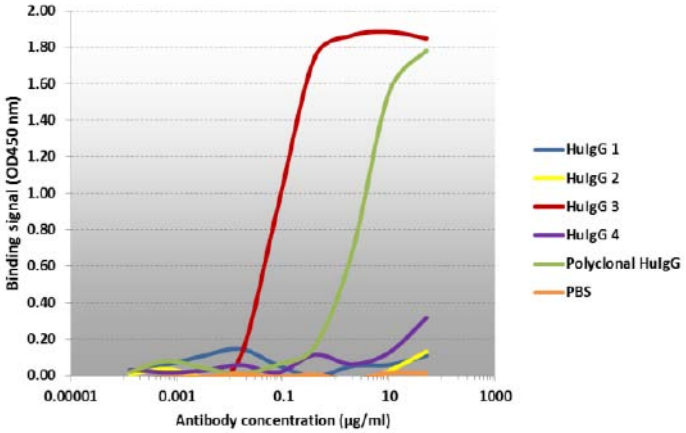


Figure 2 Example dose-response curves for human IgGs (IgG1, IgG2, IgG3, IgG4, and polyclonal IgG) in Capture ELISA using Biotin Anti-IgG3 (Hu) as the capturing agent. The antibody samples have a purity of approximately 98-99%.

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.

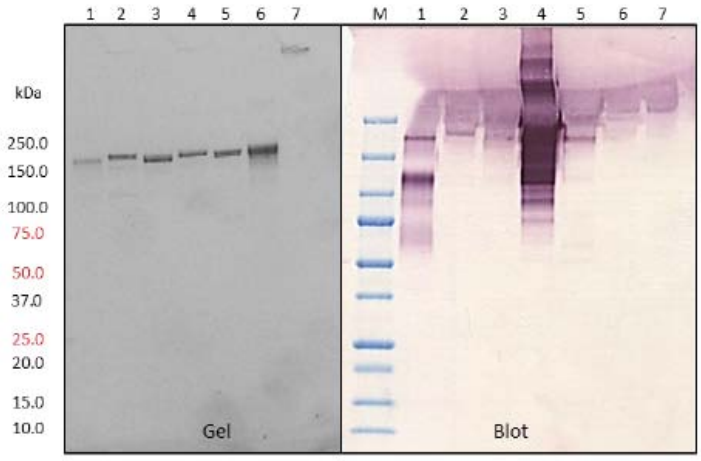
1. Run the protein sample(s) of interest by SDS PAGE under non-reducing conditions, then transfer the separated proteins onto an appropriate membrane (for example, by electroblotting).

   Note: When protein samples are run under reducing conditions, we have observed poor to no binding with Biotin Anti-IgG3 (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-IgG3 (Hu), 1 µg/mL in buffer.
4. Detect bound Biotin Anti-IgG3 (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, Biotin Anti-IgG3 (Hu) can be used in Western blot for the specific detection of human IgG3 antibodies. See Figure 3.



M All Blue Precision Plus Protein marker (BioRad)
1. Polyclonal HuIgG
2. Polyclonal HuIgG 1
3. Polyclonal HuIgG 2
4. Polyclonal HuIgG 3
5. Polyclonal HuIgG 4
6. Polyclonal HuIgA
7. Polyclonal HuIgM

Figure 3 Western blot analysis of different human antibodies (non-reduced) using Biotin Anti-IgG3 (Hu). Different human antibody samples have a purity of approximately 98–99%.

Label-free and real-time binding assays

CaptureSelect™ Biotin Conjugates 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 the Biotin Anti-IgG3 (Hu) Conjugate for use as capturing agents to measure interactions with human IgG3 antibodies.

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 human IgG3 kappa or lambda antibodies (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

CaptureSelect™ Biotin Anti-IgG3 (Hu) 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, Figure 5, and Figure 6.

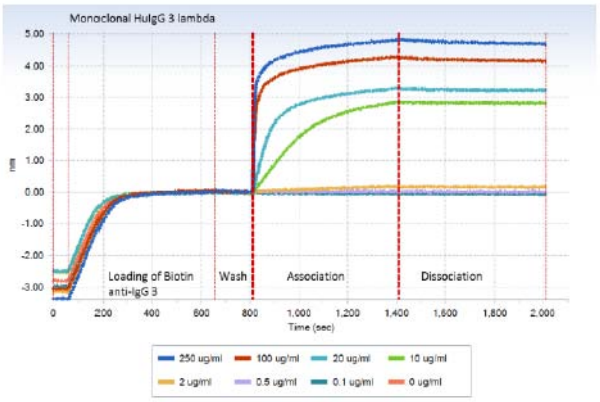


Figure 4 Binding analysis of human IgG3 lambda on Streptavidin (SA) Biosensors (Octet ™ QK system) functionalized with Biotin Anti-IgG3 (Hu) followed by association and dissociation of human IgG3 lambda at different antibody concentrations.

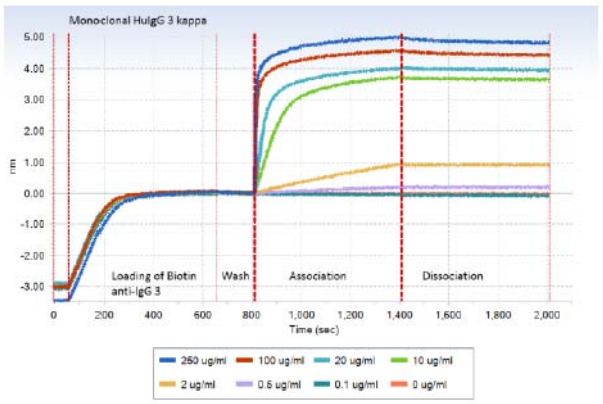


Figure 5 Binding analysis of human IgG3 kappa on Streptavidin (SA) Biosensors (Octet ™ QK system) functionalized with Biotin Anti-IgG3 (Hu) followed by association and dissociation of human IgG3 kappa at different antibody concentrations.

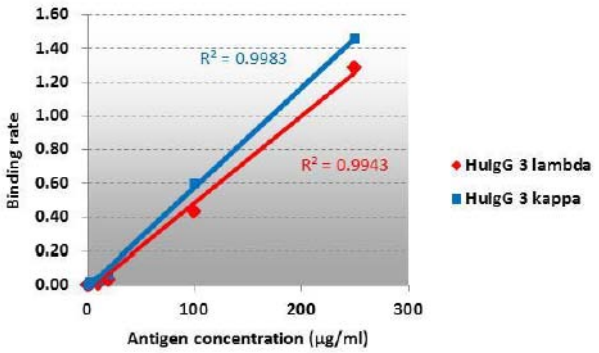


Figure 6 Example calibration curves of human IgG3 kappa and lambda on Biotin Anti–IgG3 (Hu)-functionalized Biosensors. To demonstrate the use of Biotin Anti-IgG3 (Hu) for quantitation purposes, binding rates were obtained for the first 10 seconds of association.

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

CaptureSelect™ Biotin Anti-IgG3 (Hu) Conjugate is compatible with the Biacore™ SA Sensor Chip (see Figure 7 and Table 1) and the Biacore™ Biotin CAPture Kit, which enables reversible capture of biotinylated molecules and standardized regeneration for interaction studies.

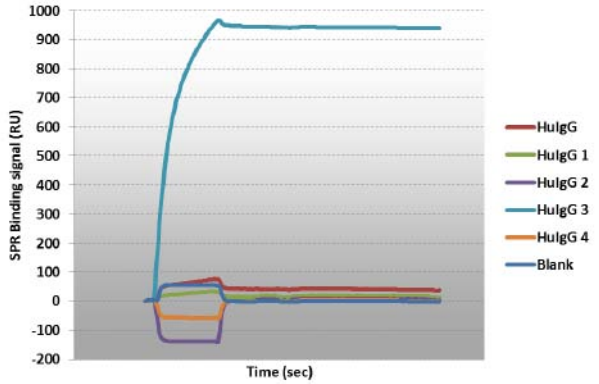


Figure 7 Association and dissociation curves of human IgG antibodies (human IgG, human IgG Fc, human IgG Fab, human IgG LC kappa, and human IgG LC lambda) on Biacore™ Sensor Chips SA (Biacore™ 3000 system) functionalized with Biotin Anti-IgG3 (Hu).

Table 1 Relative binding selectivity for a diverse set of antibodies on a Biotin Anti-IgG3 (Hu)-functionalized Biacore™ sensor chip SA (Biacore™ 3000 system). The Biotin Anti-IgG3 (Hu) format prevents cross-binding with IgG antibodies from various non-primate species such as mouse, rat, goat, and bovine.

IgG subclasses (mono- or polyclonal)	Binding [1]	IgG fragments/isotypes (polyclonal)	Binding [1]
Human IgG1 kappa	–	Human IgG Fc (hinge)	+
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

Ordering information

CaptureSelect™ Biotin Conjugates	Catalog number
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-IgG-Fc PK (pharmacokinetics)	7103322100 (100 µg) 7103322500 (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)
Anti-Factor VIII	7102862100 (100 µg) 7102862500 (500 µg)
Anti-Factor IX	7103002100 (100 µg) 7103002500 (500 µg)
Anti-FSH	7103180100 (100 µg) 7103180500 (500 µg)
Anti-hGH	7103160100 (100 µg) 7103160500 (500 µg)
Anti-GCSF	7103130100 (100 µg) 7103130500 (500 µg)
Anti-C-Tag	7103252100 (100 µg) 7103252500 (500 µg)
Anti-Insulin	7103362100 (100 µg) 7103362500 (500 µg)
Anti-EPO	7103372100 (100 µg) 7103372500 (500 µg)

For more information

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

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- Worldwide contact telephone numbers
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  - 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.

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