

CaptureSelect™ Biotin Anti-IgM Conjugate

Catalog Number 7102892100 and 7102892500

Pub. No. MAN0010063 Rev. B.0

Cat. no.	Quantity	Contents	Storage conditions
7102892100	100 µg	1 mg/mL protein in PBS, pH 7.4 (no preservatives added)	<ul style="list-style-type: none"> 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)
7102892500	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-IgM Conjugate consists of a 14 kDa Llama antibody fragment (affinity ligand) that specifically binds to the μ chain of human, rat, and mouse IgM antibodies (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-IgM format allows you to:

- **Detect, quantify, and characterize** — All human, rat, and mouse IgM antibodies (independent of the type of light chain), recombinant IgM monoclonal antibodies, and total IgM in human plasma and/or serum samples.
- **Avoid cross-binding** — Biotin Anti-IgM does not cross-bind with other isotypes such as IgG, IgA, IgD, and IgE, free light chains, or with IgM from bovine sources such as FCS.

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).

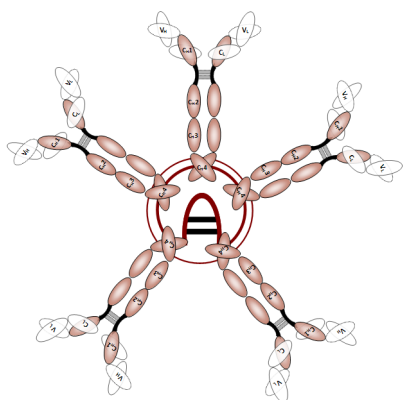


Figure 1 Representation of an IgM antibody. The μ chain (in brown) is recognized by Biotin Anti-IgM.

Binding selectivity: Biotin Anti-IgM

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 IgM Fab	-
	Human IgM Fc	✓
	Free human LC kappa	-
	Free human LC lambda	-
Ab species	Mouse IgM	✓
	Rat IgM	✓
	Bovine IgM	-

[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 – Human isotype-specific antibodies conjugated to HRPO, such as those from Jackson ImmunoResearch and 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 samples that contain human IgM. Add 100 µL/well to the Biotin Anti-IgM-functionalized plates. Let sit for 1 hour at room temperature.
 3. Use commercially available detection antibodies to detect bound antibody molecules.

- Use TMB/H₂O₂-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-IgM can be used as a capturing agent in highly sensitive assays to detect and quantitate human, rat, and mouse IgM antibodies, without cross-binding with other isotypes such as IgG, IgA, IgD, and IgE, or IgM from bovine sources such as FCS. You can detect captured antibody molecules using commercially available secondary antibody reagents. See Figure 2.

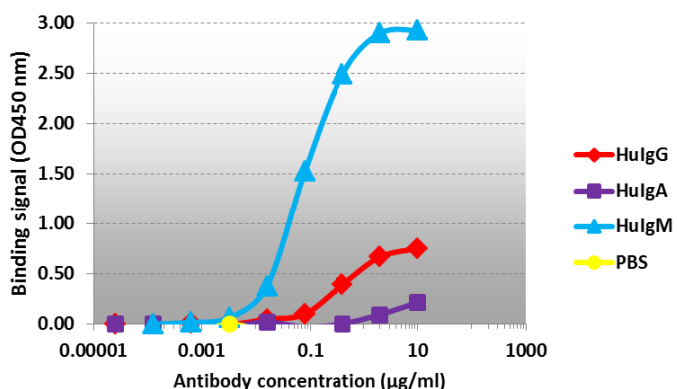


Figure 2 Example dose-response curves for polyclonal human IgM, IgG, and IgA antibodies in Capture ELISA using Biotin Anti-IgM as the capturing agent. The polyclonal samples have a purity of approximately 97%.

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.
- 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-IgM in Western blot applications.
 - Block the membrane for 1 hour at room temperature with 2% (w/v) skimmed milk in PBS.
 - Incubate the blocked membrane with Biotin Anti-IgM, 1 µg/mL in buffer.
 - Detect bound Biotin Anti-IgM using streptavidin-AP conjugate, 1:2000 in buffer.
 - 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 IgM antibodies, without cross-binding with other isotypes such as IgG and IgA, or IgM from bovine sources such as FCS. See Figure 3.

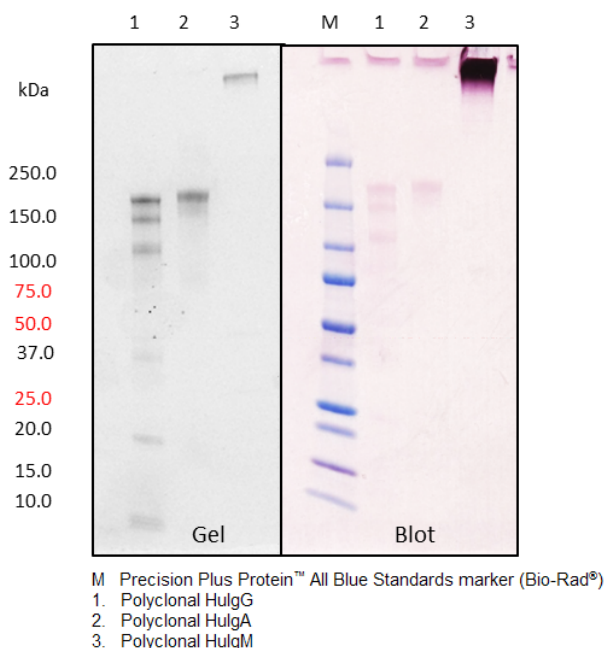


Figure 3 Western blot analysis of different polyclonal human IgM, IgG, and IgA antibodies (non-reduced) using Biotin Anti-IgM. The polyclonal samples have a purity of approximately 97%.

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 IgM antibodies (including mouse and rat IgM). Because the epitope resides on the heavy chain of IgM (µ chain), the interaction with IgM antibodies is not affected by free light chains that may be present in feedstock materials.

Bio-layer interferometry (BLI) guidelines for use

Note: Use the recommended materials or their equivalents.

- 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.
- 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.
- (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.

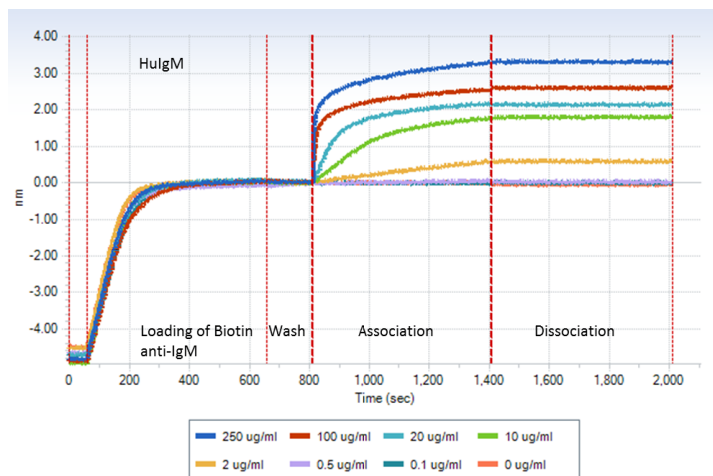


Figure 4 Binding analysis of human IgM demonstrates ForteBio™ Streptavidin (SA) Biosensors (Octet™ QK system) functionalized with Biotin Anti-IgM followed by association and dissociation of human IgM samples at different antibody concentrations.

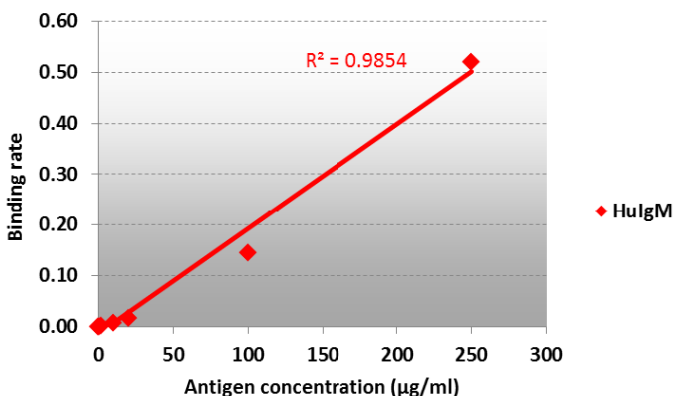


Figure 5 Example calibration curve of human IgM on Biotin Anti-IgM-functionalized biosensors. To demonstrate the use of Biotin Anti-IgM for quantitation purposes, binding rates were obtained for the first 10 seconds of association.

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.

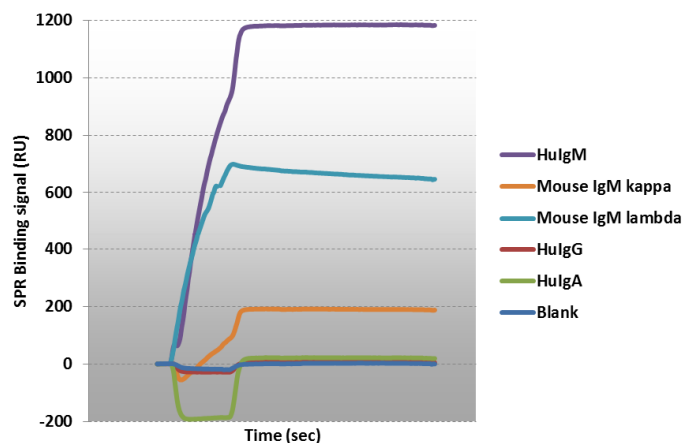


Figure 6 Association and dissociation curves of Ig antibodies (polyclonal human IgM, polyclonal human IgG, polyclonal human IgA, mouse IgM kappa, and mouse IgM lambda) on Biacore™ Sensor Chips SA (Biacore™ 3000 system) functionalized with Biotin Anti-IgM.

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

Ig subclasses (mono- or polyclonal)	Binding ^[1]	Ig species (polyclonal)	Binding ^[1]
Human IgG1	-	Human IgG	-
Human IgG2	-	Human IgM	++
Human IgG3	-	Human IgA	-
Human IgG4	-	Human IgD	-
Human IgA1	-	Human IgE	-
Human IgA2	-	Bovine IgG	-
Human secretory IgA	-	Bovine IgM	-
Human IgM Fc	++	Rat IgM	++
Human IgM Fab	-	Mouse IgM	++

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

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

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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.

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