CaptureSelect™ N-ethyl biotin conjugate: anti-CD4 and anti-CD8

Catalog Numbers 7113762100 and 7113772100

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Product description

The CaptureSelect™ N-ethyl Biotin anti-CD4 Conjugate and CaptureSelect™ N-ethyl Biotin anti-CD8 Conjugate products are used for the isolation of CD4-positive or CD8-positive cells from human whole blood, bone marrow, buffy coat, mononuclear cells, or tissue digests. When coupled to Dynabeads™ M-450 Streptavidin Beads, the conjugate enables magnetic purification and separation of the positive cell subset.

The presence of the N-ethyl group on the biotin molecule in the conjugate provides a less-stringent binding to the streptavidin molecule than the d-biotin that is added to the reaction. This competitive binding allows the release of the streptavidin bead from the biotin conjugate, providing bead-free isolation of the positive cell subset with high yield and purity. The isolated cells can be used directly in downstream applications, such as flow- and surface-plasmon resonance cytometry.

Features of the product include:

- High specificity for human CD4 or CD8 alpha chain
- N-ethyl biotin group, enabling bead-free isolation using d-biotin
- Animal-free origin

Each product provides sufficient volume for isolation of 50 × 10^7 of target cells.

Note: This protocol is based on a sample volume of 1 mL (1 × 10^7) of CD4 or CD8 target cells, but provides additional information for scaling to 50 mL (50 × 10^7) of target cells. The volumes and dilutions that are provided in this protocol can be used as a starting point to optimize for different target cell quantities.

Contents and storage

Item	Cat. No.	Amount	Storage
CaptureSelect™ N-ethyl Biotin anti-CD4 Conjugate	7113762100	100 µL (1mg/mL)	–20°C upon receipt
CaptureSelect™ N-ethyl Biotin anti-CD8 Conjugate	7113772100		

Note: The 100 µL (1mg/mL) of conjugate is sufficient for coupling to 5 vials of Dynabeads™ M-450 Streptavidin Beads (20 µg/mL of conjugate per 5 mL of magnetic beads). Aliquot the conjugate into 5 batches of 20 µL (20 µg/mL) to prevent freeze/thaw cycles.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Dynabeads™ M-450 Streptavidin Beads, 5mL, or equivalent 5 mL is sufficient for binding of 20 µg of the CaptureSelect™ anti-CD4 or anti-CD8 conjugate.	2850000005
Magnetic stands—See the following sections for the magnet sizes required: "Magnetic bead preparation volumes and materials" on page 2 and "Isolation and release volumes and materials" on page 3.	
• DynaMag™-5 Magnet • DynaMag™-15 Magnet • DynaMag™–50 Magnet	• 12303D • 12301D • 12302D
Pierce™ Biotin (for release buffer)	29129
Cole-Parmer™ Stuart™ Digital Tube Roller or equivalent	Fisher Scientific™ 11426498
Tubes: 5, 15, and 50 mL tubes	MLS
Phosphate buffered saline (PBS) (for isolation buffer)	MLS
Human serum albumin (HSA) or bovine serum albumin (BSA), 1% (for isolation and release buffers)	MLS
NaOH (for release buffer pH adjustment)	MLS

Prepare isolation and release buffers

Prepare fresh buffers before each isolation.

1. To prepare the isolation buffer, combine PBS and HSA or BSA for a final concentration of 1% HSA/BSA in PBS.
2. To prepare the 5mM d-biotin release buffer, dissolve 1 g of Pierce™ Biotin in 830 mL of isolation buffer. Adjust to pH 7.05 using NaOH solution.

Magnetic bead preparation volumes and materials

Table 1 Wash and couple the Dynabeads™ M-450 Streptavidin Beads (magnetic beads)

Step	Item	500 µL bead conjugation	5 mL bead conjugation
Wash step 2	Tube size	5 mL	15 mL
Wash step 2	Dynabeads™ M-450 Streptavidin Beads	500 µL (100 µL is required for isolation of 1×10^7 cells)	5 mL
Wash step 3	Isolation buffer	1 mL	5 mL
Wash step 4 Couple step 3	Magnetic stand	DynaMag™-5 Magnet 1 minute	DynaMag™-15 Magnet 1 minute
Wash step 6	Isolation buffer	500 µL	5 mL
Couple step 1	CaptureSelect™ anti-CD4 conjugate CaptureSelect™ anti-CD8 conjugate	20 µL (10x diluted) [1]	20 µL
Couple substep 5a	Isolation buffer	1 mL	5 mL
Couple step 7	Isolation buffer	500 µL	5 mL

[1] Prepare a 10X dilution of the conjugate in isolation buffer to allow accurate pipetting of the conjugate.

Wash the magnetic beads

This procedure is for preparing 500 µL of washed magnetic beads. 100 µL is required for isolation of 1×10^7 cells. For information on scaling to larger volumes, see "Magnetic bead preparation volumes and materials" on page 2.

1. Resuspend the magnetic beads: Vortex for >30 seconds, or tilt and rotate on a digital tube roller for 5 minutes.
2. Transfer 500 µL of the magnetic beads to a 5-mL tube.
3. Add 1 mL of isolation buffer, then resuspend the beads by gentle vortexing.
4. Place the tube in a DynaMag™-5 Magnet stand for 1 minute.
5. Discard the supernatant, then remove the tube from the magnetic stand.
6. Resuspend the washed beads in 500 µL of isolation buffer.

Couple the ligand to the magnetic beads

1. Add 20 µL (10x diluted) of the CaptureSelect™ anti-CD4 or anti-CD8 conjugate (ligand) to the washed magnetic beads.

   Note: Prepare a 10X dilution of the conjugate in isolation buffer to allow accurate pipetting of the conjugate.
2. Incubate for 30 minutes at room temperature (~20 °C) with tilting and rotation (45 rpm).
3. Place the tube in a magnetic stand for 1 minute.
4. Remove and discard the supernatant.
5. Wash the ligand-coupled beads:
   a. Remove the tube from the magnetic stand, then add 1 mL of isolation buffer.
   b. Vortex for 5 seconds (1,400 rpm), then place the tube in the magnetic stand for 1 minute.
   c. While the tube is still in the magnet, carefully remove and discard the supernatant.
6. Remove the tube from the magnetic stand.
7. Resuspend the washed, ligand-coupled magnetic beads in 500 µL of isolation buffer.

Isolation and release volumes and materials

Table 2 Isolation and release volumes and materials

Step	Item	1×10^7 cells	10×10^7 cells	50×10^7 cells
Isolate step 1	Cell solution volume	1 mL	10 mL	2 × 25 mL
	Tube size	5 mL	50 mL	2 × 50-mL
Isolate step 2	Ligand-coupled magnetic beads	100 µL	1 mL	2 × 2.5 mL
Isolate step 5 and substep 7b Release step 4 and substep 6b	Magnetic stand	DynaMag™-5 Magnet 1 minute	DynaMag™–50 Magnet 1 minute	DynaMag™–50 Magnet 1 minute
Isolate substep 7a	Isolation buffer	1 mL	10 mL	2 × 25 mL
Release step 1	Release buffer	2 mL	20 mL	2 × 50 mL
Release step 5	Tube size	5 mL	50 mL	2 × 50 mL
Release step 6	Isolation buffer	1 mL	10 mL	2 × 25 mL

Prepare the target cells

1. Using appropriate methods, determine cell concentration and the percentage of CD4 or CD8 target cells.
2. Dilute the peripheral blood mononuclear cell (PBMC) samples in isolation buffer for a concentration of 1×10^7 target cells/mL.

   If the volume of 1×10^7 target cells/mL is <1 mL, bring to 1 mL with isolation buffer.

   If the volume of 1×10^7 target cells/mL is >1 mL, scale up remaining volumes accordingly.

Isolate the CD4- or CD8-positive cells

This procedure is for isolation of CD-4 or CD-8-positive cells from 1 mL of target cells (1×10^7). For information on scaling to larger volumes, see Table 2.

1. Transfer 1 mL of cells (1×10^7 target cells) to a 5 mL tube.
2. Add 100 µL of ligand-coupled magnetic beads.
3. Resuspend the magnetic beads: Vortex for >30 seconds, or tilt and rotate on a digital tube roller for 5 minutes.
4. Incubate for 10 minutes at room temperature (~20 °C) with tilting and rotation (45 rpm).
5. Place the tube in a DynaMag™-5 Magnet stand for 1 minute to form the cell-bead pellet.
6. While the tube is still in the magnetic stand, carefully remove and discard the supernatant.
7. Wash the bead-bound CD4- or CD8-positive cells:
   a. Remove the tube from the magnetic stand, then add 1 mL of isolation buffer.
   b. Vortex for 5 seconds (1,400 rpm), then place the tube in the magnetic stand for 1 minute.
   c. While the tube is still in the magnet, carefully remove and discard the supernatant.
8. Repeat step step 7 to wash the beads 2 additional times.

IMPORTANT! Adequate washing is critical to obtain a high purity of isolated cells.

Release the CD4- or CD8-positive cells

1. Resuspend the bead-cell pellet in 2 mL of release buffer by vortexing.
2. Incubate the tube for 20–60 minutes at room temperature (~20 °C) with tilting and rotation (45 rpm).

   Note: Release of cells is time dependent. Optimize the time for your application.
3. Pipet up and down 10 times to release the cells. Avoid foaming.
4. Place the tube in the DynaMag™-5 Magnet stand for 1 minute.
5. While the tube is still in the magnetic stand, transfer the supernatant containing the bead-free CD4 or CD8-positive cells to a new 5-mL harvest tube.
6. Wash the beads to obtain the remaining bead-free CD4- or CD8-positive cells:
   a. Remove the tube from the magnetic stand, then add 1 mL of isolation buffer.
   b. Vortex for 5 seconds (1,800 rpm), then place the tube in the magnetic stand for 1 minute.
   c. While the tube is still in the magnetic stand, carefully remove the supernatant containing the bead-free CD4- or CD8-positive cells, then add it to the harvest tube from step 5.

7. Remove the tube containing magnetic beads from the magnetic stand, then discard the tube.

8. Store the CD4- or CD8-positive cells at 2°C–8°C until further use in downstream applications.

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  - 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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For more information

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

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	12 July 2021	New document for CaptureSelect™ N-ethyl Biotin anti-CD4 Conjugate and CaptureSelect™ N-ethyl Biotin anti-CD8 Conjugate.