Automated Dynabeads™-Based Cell Capture using the KingFisher™ Apex or KingFisher™ Flex Purification Systems

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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

The Invitrogen™ Dynabeads™ superparamagnetic beads target CD3+, CD4+, CD8+, CD14+, CD19+ (Pan B), and CD15+ leukocyte subpopulations, enabling easy isolation or depletion of human immune cells directly from whole blood or peripheral blood mononuclear cells (PBMCs). The cell isolation or depletion process is simple. The sample is mixed with the beads for targeted cell capture. After a short incubation, the bead-bound cells are separated by the magnetic head of a KingFisher™ instrument. The sample is now depleted of the target cells. After an optional wash step, the bead-bound cells are ready for further downstream analysis, including lysis and processing for protein or nucleic acid analysis.

This guide describes automated magnetic bead-based isolation or depletion of human immune cells directly from whole blood or PBMCs using the KingFisher™ Apex or the KingFisher™ Flex Purification System.

The entire automated protocol is completed in 40 minutes, allowing up to 96 samples to be processed simultaneously in a single run.

Contents and storage

Table 1 Dynabeads™ superparamagnetic beads

Item	Cat. No.	Amount	Total PBMC capacity	Storage
Dynabeads™ CD3	11152D	2 mL	8×10^8	2–8°C
	11151D	5 mL	2×10^9	
Dynabeads™ CD4	11146D	2 mL	8×10^8	
	11145D	5 mL	2×10^9	
Dynabeads™ CD8	11148D	2 mL	8×10^8	
	11147D	5 mL	2×10^9	
Dynabeads™ CD14	11150D	2 mL	8×10^8	
	11149D	5 mL	2×10^9	
Dynabeads™ CD19 Pan B	11144D	2 mL	2×10^9	
	11143D	5 mL	5×10^9	
Dynabeads™ CD15	11138D	2 mL	8×10^8	
	11137D	5 mL	2×10^9	

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.

IMPORTANT! Use only barcoded plates and tip combs with the KingFisher™ Apex instrument. Non-barcoded deep-well plates or tip combs will increase processing times, may damage the system, void the warranty, and reduce performance.

Item	Source
Instruments	
KingFisher™ Apex with 96 Deep-Well Head	5400930
KingFisher™ Flex with 96 Deep-Well Head	5400630
Equipment	
Laboratory mixer, vortex, or equivalent	MLS
Single and multichannel adjustable pipettors (1 µL–1 mL)	MLS
Plates, combs, plate seals, and pipetting consumables	
KingFisher™ 96 Deep-Well Tip Comb, Barcoded	97002534B
KingFisher™ 96 Deep-Well Plates, Barcoded	95040450B
KingFisher™ 96 Deep-Well Plates	95040450
KingFisher™ 96 Deep-Well Tip Comb	97002534
MicroAmp™ Adhesive Film Applicator	4333183
MicroAmp™ Clear Adhesive Film	4306311
Pipette tips (10 µL–1 mL), sterile	MLS
Reagents	
Isolation Buffer: Calcium and magnesium-free phosphate buffered saline (PBS) with 0.1% BSA and 2 mM EDTA, pH 7.4[1]	MLS

[1] BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate.

Procedural guidelines

- For cell depletion, a 30-minute incubation is recommended.
- For positive isolation of cells, the incubation time can be reduced to 20 minutes.

  Note: Longer incubation times may lead to reduced purity.
- For positive cell isolation, it is recommended to add an additional wash step after cell capture in the script.
- Incubation can be conducted at a reduced temperature on the KingFisher™ Apex instrument.

  Note: Cell capture at lower temperatures can increase purity while decreasing capture efficiency.
- For some targets (for example CD3), cell capture efficiency from whole blood can be increased by pre-processing the sample, such as washing. Refer to the product-specific user manual for guidance. See “Related documentation” on page 4.
- If cell depletion is not sufficient, the quantity of beads can be increased up to double the recommended volume to achieve higher depletion efficiency. This is particularly relevant for CD3 cell depletion, or for cell depletion at lower temperature.

Set up the instrument

The appropriate script must be installed on the instrument before first use. The scripts are optimized for cell depletion, and 500 µL PBMC suspension is used as starting sample. The protocols are scalable from 100 µL–1 mL.

- Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well).
- Ensure that the instrument is set up with the correct heat block (96 deep-well) when changing the script to cooled processing.

1. On the product web page (at thermofisher.com, search by catalog number), scroll to the **Documents & Downloads** section.
2. Locate and download the appropriate file for your instrument, then install the script onto your instrument.

   Note: The scripts serve as proof-of-concept and can be modified to meet specific user needs.
3. Refer to your instrument user guide or contact Technical Support at http://thermofisher.com/support for detailed instructions on script installation.

Prepare magnetic beads

Vortex the magnetic beads in the vial for 30 seconds, then rotate the vial for 5 minutes.

Note: Vortex and rotate the beads immediately before use to ensure optimal performance. Ensure that no beads remain adhered to the vial.

Prepare samples

Target cells can be directly captured from samples such as PBMC suspensions or whole blood.

1. Adjust PBMC suspension to a concentration of 1×10^7 cells/mL using Isolation Buffer.

   Note: For Dynabeads™ CD19 Pan B, adjust the PBMC suspension to 2.5×10^7 cells/mL.
2. Dilute whole blood 1:2 in Isolation Buffer for optimal capture efficiency.

Automated workflow for cell depletion

Use the following automated workflow as a starting point. Further optimization may be required, depending on sample type, downstream application, or when working with different volumes. For recommendations for cell depletion, see “Procedural guidelines” on page 2.

1 Set up the processing plates

1. Label the side of four KingFisher™ 96 Deep-Well Plates according to the Plate IDs in Table 2.
2. Set up the processing plates according to the following table.

Table 2 Processing plate set-up

Plate ID	Plate loading position[1]	Reagents	Volume per well	Plate type
Tip	1	Place a Tip Comb into an empty deep-well plate		Deep-well plate
Beads	2	Isolation Buffer	500 µL	
		Beads	12.5 µL	
Sample	3	Cell Suspension	500 µL	
Captured Cells	4	Isolation Buffer	100 µL	

[1] Position on the instrument.

3. Proceed to “Process samples on the instrument”.

2 Process samples on the instrument

1. Select the appropriate script on the instrument.
2. Start the run, then load the prepared processing plates in their positions when prompted by the instrument.

   Note: Once all four plates are loaded, the instrument will start processing.

   IMPORTANT! When loading plates, ensure that the A1 position of the plate aligns with the A1 position indicated on the instrument turntable.
3. At the end of the run, remove the Sample Plate from the instrument, then proceed with the desired downstream analysis, or cover the plate with MicroAmp™ Clear Adhesive Film.

Automated workflow for positive cell isolation

Use the following automated workflow as a starting point. Further optimization may be required. For recommendations for positive cell isolation, see “Procedural guidelines” on page 2.

1 Set up the processing plates

1. Label the side of five deep-well plates according to the Plate IDs in Table 3.
2. Set up the processing plates according to the following table.

Table 3 Processing plate set-up

Plate ID	Plate loading position[1]	Reagents	Volume per well	Plate type
Tip Comb	1	Place a Tip Comb into an empty deep-well plate		Deep-well plate
Beads	2	Isolation Buffer	500 µL	
		Beads	12.5 µL	
Sample	3	Cell Suspension	500 µL	
Wash	4	Isolation Buffer	600 µL	
Captured Cells	5	Isolation Buffer	100 µL	

[1] Position on the instrument.

3. Proceed to “Process samples on the instrument”.

2 Process samples on the instrument

1. Select the appropriate script on the instrument.

   Note: Positive cell isolation requires updating the script. The suggested wash settings are 1 minute with slow mixing in 600 µL Isolation Buffer.

2. Start the run, then load the prepared processing plates in their positions when prompted by the instrument.

   Note: Once all five plates are loaded, the instrument will start processing.

   IMPORTANT! When loading plates, ensure that the A1 position of the plate aligns with the A1 position indicated on the instrument turntable.

3. At the end of the run, remove the Captured Cells Plate from the instrument, then proceed with the desired downstream analysis, or cover the plate with MicroAmp™ Clear Adhesive Film.

For downstream analysis, the beads can be resuspended directly in the desired lysis buffer instead of isolation buffer. Optimize the volume of lysis buffer as needed.

Related documentation

Table 4 Product-specific user manuals

Document	Publication number	Cat. No.
Dynabeads™ CD3 User Guide	MAN1000848	11152D, 11151D
Dynabeads™ CD4 User Guide	MAN1000849	11146D, 11145D
Dynabeads™ CD8 User Guide	MAN1000850	11148D, 11147D
Dynabeads™ CD14 User Guide	MAN1000851	11150D, 11149D
Dynabeads™ CD19 Pan B User Guide	MAN1000852	11144D, 11143D
Dynabeads™ CD15 User Guide	MAN1000853	11138D, 11137D

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

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- Order and web support
- Product documentation
  - 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

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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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	3 January 2025	New document for Automated Dynabeads™ -Based Cell Capture using the KingFisher™ Apex or KingFisher™ Flex Purification System.