Automated Dynabeads™-Based Isolation of Human Epithelial Cells using the KingFisher™ Apex Purification System

Catalog Numbers 16102, 16103D

Pub. No. MAN1000760 Rev. A

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™ Epithelial Enrich are superparamagnetic beads coupled to a monoclonal mouse anti-human EpCAM antibody. The beads are designed to isolate epithelial circulating tumor cells (CTCs) from liquid biopsy samples. The isolation process is simple. The sample is mixed with the beads for targeted cell capture. After a short incubation and subsequent washing, the bead-bound cells are separated by the magnetic head of a KingFisher™ instrument. The bead-bound cells are then ready for further downstream analysis, including lysis and processing for protein or nucleic acid analysis.

This guide describes automated magnetic bead-based isolation of human epithelial cells from peripheral blood mononuclear cells (PBMCs) or whole blood using the KingFisher™ Apex Purification System.

The automated protocol is completed in 45 minutes, allowing up to 96 samples to be processed simultaneously in a single run.

Contents and storage

Table 1 Dynabeads™ Epithelial Enrich

Item	Cat. No.	Amount	Total PBMC capacity	Whole blood capacity	Storage
Dynabeads™ Epithelial Enrich	16103D	2 mL	8×10^8	80 mL	2–8°C
	16102	5 mL	2×10^9	200 mL	

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
Instrument	
KingFisher™ Apex with 96 Deep-Well Head	5400930
Equipment	
Laboratory mixer, vortex, or equivalent	MLS
Single and multichannel adjustable pipettors (1 µL–1 mL)	MLS

Item	Source
Plates, combs, plate seals, and pipetting consumables	
KingFisher™ 96 Tip Comb for Deep-Well Magnets, Barcoded	97002534B
KingFisher™ 96 Deep-Well Plates, Barcoded	95040450B
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.

Set up the instrument

Ensure that the instrument is set up for processing with the magnetic head (96 deep-well) and heat block (96 deep-well).

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 script file for your instrument and install it onto the instrument.

   Note: The script serves as proof-of-concept and can be modified to meet specific user needs.
3. Refer to your KingFisher™ 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 PBMC suspensions.

Note: Keep anti-coagulated whole blood containing EDTA or ACD on ice.

Set up the processing plates

1. Label the side of five 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 Dynabeads™ Epithelial Enrich	175 µL 25 µL	
Wash	3	Isolation Buffer	500 µL	
Sample	4	PBMC suspension	1000 µL	
Elution	5	Isolation Buffer	100 µL	

[1] Position on the instrument.

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

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 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 Elution Plate from the instrument. If the samples are not processed immediately, 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 3 Product-specific user manual

Document	Publication number	Cat. No.
Dynabeads™ Epithelial Enrich User Guide	MAN1000855	16102, 16103D

Customer and technical support

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

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments

- 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.

Revision history: Pub. No. MAN1000759 A

Revision	Date	Description
A	3 January 2025	New document for Automated Dynabeads-Based Isolation of Human Epithelial Cells using the KingFisher™ Apex Purification System.

The information in this guide is subject to change without notice.

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