EasySep™ Mouse Epithelial Cell Enrichment Kit II

Negative Selection

Catalog #19868

For processing 1 x 10^9 cells

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Description

Enrich untouched and highly purified epithelial cells from freshly dissociated mouse mammary tissues by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets non-epithelial cells for removal with biotinylated antibodies recognizing non-epithelial cell surface markers. Unwanted cells are labeled with biotinylated antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse Epithelial Cell Enrichment Cocktail	19757C.1	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS. Includes an Fc receptor blocking antibody.
EasySep™ Mouse Biotin Selection Cocktail	19153	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Dextran RapidSpheres™ 50100	50100	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

DISSOCIATION OF MOUSE MAMMARY TISSUE

Use Gentle Collagenase/Hyaluronidase (Catalog #07919) to enzymatically digest mouse mammary tissue. Refer to the associated Product Information Sheet (Document #29629) for detailed information on the recommended protocol. For more information, visit www.stemcell.com or contact us at techsupport@stemcell.com

After preparation, resuspend cells at 1 x 10^8 cells/mL in recommended medium.

Recommended Medium

HBSS with 10 mM HEPES, Without Phenol Red (Catalog #37150) containing 2% fetal bovine serum (FBS).

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse Epithelial Cell Enrichment Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.2 - 2 mL NOTE: If starting with fewer than 2 x 10^7 cells, resuspend cells in 0.2 mL.	1 x 10^8 cells/mL 0.5 - 8 mL
2	Add DNase to sample.	100 µg/mL of sample	100 µg/mL of sample
3	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Corning Catalog #38008)
4	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
5	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
7	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 3 minutes	2 - 8°C for 3 minutes
8	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	• Top up to 5 mL for samples < 1 mL • Top up to 10 mL for samples ≥ 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
10	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for another separation.	RT for 5 minutes	RT for 5 minutes
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
12	Repeat steps as indicated.	Steps 10 and 11 (for a total of 3 x 5-minute separations) Isolated cells are ready for use	Steps 10 and 11 (for a total of 3 x 5-minute separations) Isolated cells are ready for use

RT - room temperature (15 - 25°C)

* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Notes and Tips

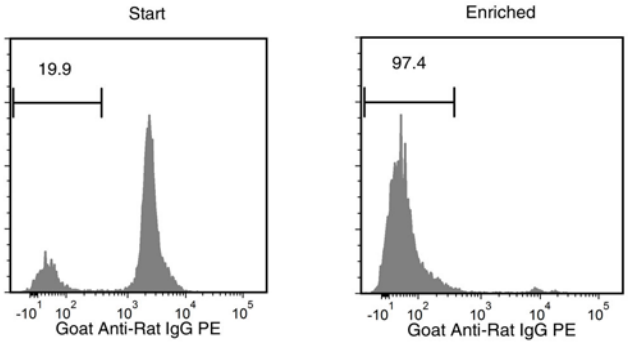
ASSESSING PURITY

For purity assessment of epithelial cells by flow cytometry use a fluorochrome-conjugated goat anti-rat IgG antibody, which will label any residual non-epithelial cells (e.g. BioLegend Catalog #405406).

SELECTION OF MOUSE MAMMARY STEM CELLS

For further detection or selection of mouse mammary luminal, basal, and stem cells, incubate the epithelial-enriched cell preparation with antibodies specific to CD24 (e.g. Anti-Mouse CD24 Antibody, Clone M1/69, PE; Catalog #60099PE) and CD49f (e.g. Anti-Mouse CD49f Antibody, Clone GoH3, FITC; Catalog #60037FI). EpiCult™-B Mouse Medium Kit (Catalog #05610) can be used as a medium for growth and culture of mouse mammary progenitor cells.

Data



Starting with mouse mammary tissues, the epithelial cell content of the enriched fraction is typically 96.97 ± 0.54% (mean ± SD using the purple EasySep™ Magnet). In the above example, the percentages of epithelial cells in the start and final enriched fractions are 19.9% and 97.4%, respectively.