



EasySep™ Mouse Epithelial Cell Enrichment Kit

Negative Selection
Catalog #19758

For processing 1×10^9 cells



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Description

Isolate 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.

For further selection of mouse mammary stem cells, use the EasySep™ Mouse Mammary Stem Cell Enrichment Kit (Catalog #19757), which contains two additional antibodies for the selection of mammary epithelial stem cells. The EpiCult™- B Mouse Medium Kit (Catalog #05610) can be used as a medium for growth and culture of mouse mammary progenitor cells.

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.
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™ Magnetic Nanoparticles	19150	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

MONONUCLEAR CELL SUSPENSION

Prepare a mononuclear cell suspension using Collagenase/Hyaluronidase (Catalog #07912) or Gentle Collagenase/Hyaluronidase (Catalog #07919). Please refer to these products' Product Information Sheets (Document #29634 and 29629, respectively) for detailed information on the recommended protocol. For more information, please visit www.stemcell.com and download our Technical Bulletin - A Guide To Solid Tissue Dissociation (Document #29107).

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



Recommended Medium



Hanks' Balanced Salt Solution (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 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 a minimum of 0.2 mL of recommended medium	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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
4	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 15 minutes	On ice for 15 minutes
5	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	On ice for 15 minutes	On ice for 15 minutes
6	Mix Magnetic Particles.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
7	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 15 minutes	On ice for 15 minutes
OPTIONAL STEP for purity assessment		Take a small aliquot (see Assessing Purity)	Take a small aliquot (see Assessing Purity)
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	<ul style="list-style-type: none"> • 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 new 5 mL tube	Use new 14 mL tube
10	Remove the tube from the magnet and add recommended medium to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2 mL	Top up to 2 mL
	Replace the tube (without lid) into the magnet and incubate.	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.	Combine with poured-off fraction from step 8	Combine with poured-off fraction from step 8

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001) 
12	Centrifuge enriched cell suspension.	300 x g for 5 minutes	300 x g for 5 minutes
13	Discard the supernatant and resuspend the cell pellet in recommended medium to the indicated volume.	Top up to 2.5 mL	<ul style="list-style-type: none"> • 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
14	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use new 5 mL tube	Use new 14 mL tube
15	Centrifuge the enriched cell suspension.	300 x g for 5 minutes	300 x g for 5 minutes
16	Discard supernatant and resuspend cells in desired medium.	Isolated cells are ready for use	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 the following fluorochrome-conjugated antibody:

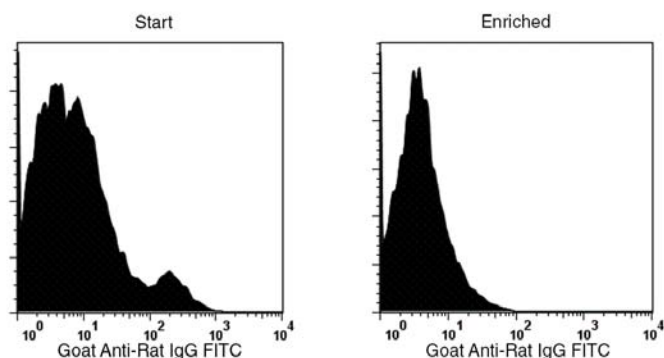
- FITC goat anti-rat IgG (e.g. Jackson ImmunoResearch Catalog #112-095-167)

FITC goat anti-rat IgG detects the primary antibodies used in the EasySep™ Epithelial Cell Enrichment Cocktail.

To prepare cells for flow cytometry analysis:

1. Take a small aliquot of non-enriched cells after adding magnetic particles to sample.
2. Wash cells once by topping up the sample tube with recommended medium.
3. Centrifuge at 350 x g for 5 minutes.
4. Discard the supernatant and resuspend the cells in recommended medium.
5. Take a small aliquot of enriched cells at the end of cell separation.
6. Label both washed cell samples with a secondary antibody (e.g. FITC goat anti-rat IgG).
7. Assess purity by flow cytometry.

Data



In the above example, the percentage of hematopoietic, endothelial, and fibroblast cells in the start and enriched fractions are $10.4 \pm 0.5\%$ and $1.1 \pm 0.2\%$, respectively.

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