



## EasySep™ Rat B Cell Isolation Kit

Negative Selection

Catalog #19644

For processing  $1 \times 10^9$  cells



Scientists Helping Scientists™ | [WWW.STEMCELL.COM](http://WWW.STEMCELL.COM)

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Document #DX20711 | Version 1\_0\_0

## Description

Isolate untouched and highly purified B cells from rat splenocytes, lymph nodes, or whole blood by immunomagnetic negative selection. When using a single-cell suspension from other tissue types, this kit may require optimization.\*

- Fast, easy-to-use and column-free
- Up to 95% purity
- Isolated cells are untouched

This kit targets non-B cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with 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, cell culture, or DNA/RNA extraction.

\* If isolating rat B cells from bone marrow mononuclear cells, contact STEMCELL Technologies' Technical Support at [techsupport@stemcell.com](mailto:techsupport@stemcell.com) for recommended cell isolation and staining protocols.

## Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Rat B Cell Isolation Cocktail	19644C	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™ 50102	50102	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

### SPLEEN or LYMPH NODE

Disrupt spleen or lymph node in recommended medium. Remove aggregates and debris by passing cell suspension through a pre-wetted 70 µm mesh nylon strainer. Centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend at  $5 \times 10^7$  nucleated cells/mL in recommended medium.

If aggregation persists, pass the cell suspension through a pre-wetted 70 µm mesh nylon strainer a second time and centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at  $5 \times 10^7$  nucleated cells/mL in recommended medium.

Ammonium chloride treatment is NOT recommended when preparing the cells for separation.

Keep the cell suspension at 2 - 8°C until ready to start the cell separation protocol.

### WHOLE BLOOD

Prepare a peripheral blood mononuclear cell (PBMC) suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD\*\* (Catalog #85450/85415) cell isolation tube. If platelet removal is desired, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at  $5 \times 10^7$  nucleated cells/mL in recommended medium.

\*\* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation.



## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS (Catalog #37350) containing 2% fetal bovine serum (FBS) and 1 mM EDTA. Hanks' Balanced Salt Solution (HBSS), Modified without Ca++ and Mg++ (Catalog #37250) can be used in place of PBS. Medium should be free of Ca++ and Mg++.

## Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Rat B Cell Isolation Kit Protocol**

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 <b>EasySep™</b> (Catalog #18000)	 <b>“The Big Easy”</b> (Catalog #18001)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 <sup>7</sup> cells/mL 0.5 - 2 mL	5 x 10 <sup>7</sup> cells/mL 1 - 8 mL
	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)
2	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	25 µL/mL of sample	25 µL/mL of sample
	Mix.	No incubation, IMMEDIATELY proceed to next step	No incubation, IMMEDIATELY proceed to next step
5	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"> <li>• Top up to 5 mL for samples ≤ 2 mL</li> <li>• Top up to 10 mL for samples &gt; 2 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	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
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 3 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	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.

**Table 2. EasySep™ Rat B Cell Isolation Kit Protocol**

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002)
		5 mL tube	14 mL tube	
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 <sup>7</sup> cells/mL 0.5 - 2 mL	5 x 10 <sup>7</sup> cells/mL 1 - 8 mL	5 x 10 <sup>7</sup> cells/mL 5 - 40 mL
	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)	50 mL conical tube (e.g. Corning Catalog #352070)
2	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
3	Vortex RapidSpheres™.	30 seconds	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate (only if required).	No incubation, IMMEDIATELY proceed to next step	No incubation, IMMEDIATELY proceed to next step	RT for 5 minutes
5	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"> <li>Top up to 5 mL for samples ≤ 2 mL</li> <li>Top up to 10 mL for samples &gt; 2 mL</li> </ul>	<ul style="list-style-type: none"> <li>Top up to 25 mL for samples ≤ 10 mL</li> <li>Top up to 50 mL for samples &gt; 10 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
8	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use


RT - room temperature (15 - 25°C)

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

**Table 3. RoboSep™ Rat B Cell Isolation Kit Protocol**

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)	
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 <sup>7</sup> cells/mL 1 - 8 mL	
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	
2	Select protocol.	Rat B Cell Isolation 19644	
3	Vortex RapidSpheres™.	30 seconds	
4	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green “Run” button	
5	Unload the carousel when the run is complete.	Isolated cells are ready for use	

## Notes and Tips

### RAT STRAINS

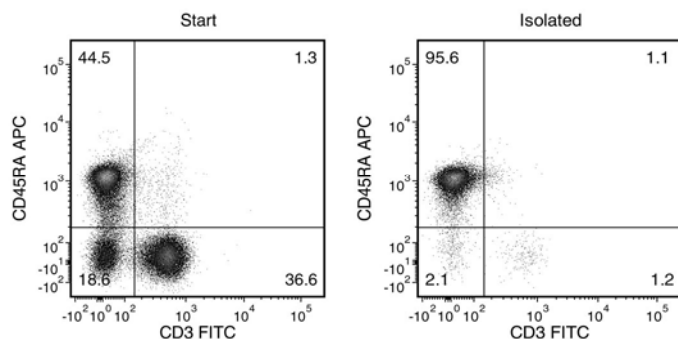
This kit has been verified for use with the Sprague Dawley strain and is expected to also be compatible with other strains.

### ASSESSING PURITY

For purity assessment of B cells by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-rat CD45RA antibody and
- Anti-rat CD3 antibody

## Data



Starting with a single-cell suspension of rat splenocytes, the B cell content (CD45RA+CD3-) of the isolated fraction is typically 94.1 ± 3.8% (mean ± SD using the purple EasySep™ Magnet). In the example above, the purities of the start and final isolated fractions are 44.5% and 95.6%, respectively.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2016 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasyEights, EasySep, RapidSpheres, RoboSep, and SepMate are trademarks of STEMCELL Technologies Inc. Lymphoprep is a trademark of AXIS-SHIELD. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.