



## EasySep™ Dead Cell Removal (Annexin V) Kit

Negative Selection

Catalog #17899

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](mailto:INFO@STEMCELL.COM) • [TECHSUPPORT@STEMCELL.COM](mailto:TECHSUPPORT@STEMCELL.COM)

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Document #DX21956 | Version 1\_0\_0

## Description

Isolate highly viable cells from cell culture or tissue preparations by immunomagnetic negative selection.

- Fast and easy-to-use
- No columns required
- Compatible across EasySep™, "The Big Easy", and EasyEights™ platforms

This kit targets phosphatidylserine on the outer leaflet of the cell membrane of apoptotic cells using Annexin V. Unwanted cells are labeled with Annexin V, 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.

## Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Dead Cell Removal (Annexin V) Cocktail	17899C	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	Protein in PBS, 0.2% BSA, and < 0.1% sodium azide.
EasySep™ Biotin Selection Cocktail	18153	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™ 50103	50103	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.

BSA - bovine serum albumin; PBS - phosphate-buffered saline

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

## Sample Preparation

Prepare a single-cell suspension in recommended medium.

## Recommended Medium



PBS containing 2% FBS and 1 mM  $\text{CaCl}_2$ .

NOTE:  $\text{CaCl}_2$  is required for optimal product performance.

## 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™ Dead Cell Removal (Annexin V) 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.	1 x 10 <sup>8</sup> cells/mL 0.1 - 2 mL NOTE: If starting with fewer than 1 x 10 <sup>7</sup> cells, resuspend cells in 0.1 mL	1 x 10 <sup>8</sup> cells/mL 0.25 - 8 mL
	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. Catalog #38008)
2	Add Dead Cell Removal (Annexin V) Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
3	Add Biotin Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample and mix.	100 µL/mL of sample No incubation, IMMEDIATELY move to next step	100 µL/mL of sample No incubation, IMMEDIATELY move to next step
6	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
7	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™ Dead Cell Removal (Annexin V) Kit Protocol**

		<b>EASYSEP™ MAGNETS</b>	
<b>STEP</b>	<b>INSTRUCTIONS</b>	<b>EasyEights™ (Catalog #18103)</b>	
		<b>5 mL tube</b>	<b>14 mL tube</b>
<b>1</b>	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 <sup>8</sup> cells/mL 0.1 - 2 mL NOTE: If starting with fewer than 1 x 10 <sup>7</sup> cells, resuspend cells in 0.1 mL	1 x 10 <sup>8</sup> cells/mL 1 - 8 mL
	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. Catalog #38008)
<b>2</b>	Add Dead Cell Removal (Annexin V) Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
<b>3</b>	Add Biotin Selection Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
<b>4</b>	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
<b>5</b>	Add RapidSpheres™ to sample and mix.	100 µL/mL of sample No incubation, IMMEDIATELY move to next step	100 µL/mL of sample No incubation, IMMEDIATELY move to next step
<b>6</b>	Add recommended medium to top up 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 10 minutes	RT for 10 minutes
<b>7</b>	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

RT - room temperature (15 - 25°C)

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

## Notes and Tips

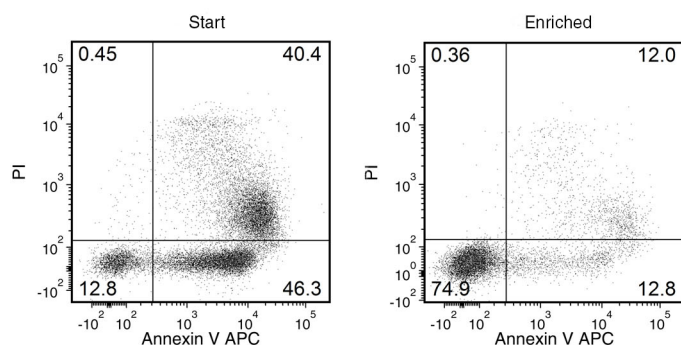
### ASSESSING PURITY

For viability assessment of cells by flow cytometry use the following:

- Fluorochrome-conjugated Annexin V antibody, and
- Propidium Iodide (Catalog #75002)

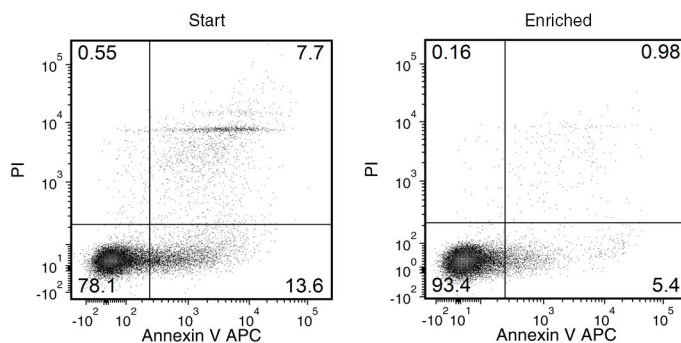
## Data

### A Human PMNCs with High Percentage of AnnexinV+ Cells



Starting with human polymorphonuclear cells (PMNCs) cultured overnight, the live cell content (AnnexinV<sup>-</sup>/PI<sup>-</sup>) of the enriched fraction is typically  $69.7 \pm 12.5\%$  (mean  $\pm$  SD using the purple EasySep™ Magnet). In the above example, the percentages of live cells in the start and final enriched fractions are 12.8% and 74.9%, respectively.

### B Mouse Splenocytes with Low Percentage of AnnexinV+ Cells



Starting with 24- to 48-hour-old mouse splenocytes, the live cell content of the enriched fraction is typically  $79.8 \pm 11.4\%$  (mean  $\pm$  SD using the purple EasySep™ Magnet). In the above example, the percentages of live cells in the start and final enriched fractions are 78.1% and 93.4%, 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 © 2017 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasySep, EasyEight, and RapidSpheres are trademarks of STEMCELL Technologies Canada Inc. 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.