EasySep™ Dead Cell Removal (Annexin V) Kit

For processing 1 x 10^9 cells

Catalog #17899

Negative Selection

Document #1000000718 | Version 01

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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% fetal bovine serum (FBS) and 1 mM $CaCl_2$. To prepare 1 L of recommended medium, add 110.98 mg of $CaCl_2$ and 20 mL of FBS to 980 mL of PBS; mix thoroughly.

NOTE: $CaCl_2$ is required for product performance. Keep buffer sterile.

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	 **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 0.1 - 2 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL	1 x 10^8 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.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
3	**Add Biotin Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	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	• Top up to 5 mL for samples ≤ 2 mL • Top up to 10 mL for samples > 2 mL
	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.

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Table 2. EasySep™ Dead Cell Removal (Annexin V) Kit Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.1 - 2 mL NOTE: If starting with fewer than 1 x 10^7 cells, resuspend cells in 0.1 mL	1 x 10^8 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)
2	Add Dead Cell Removal (Annexin V) Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
3	Add Biotin Selection Cocktail to sample. NOTE: Do not vortex cocktail.	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 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 ≤ 2 mL • Top up to 10 mL for samples > 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
7	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

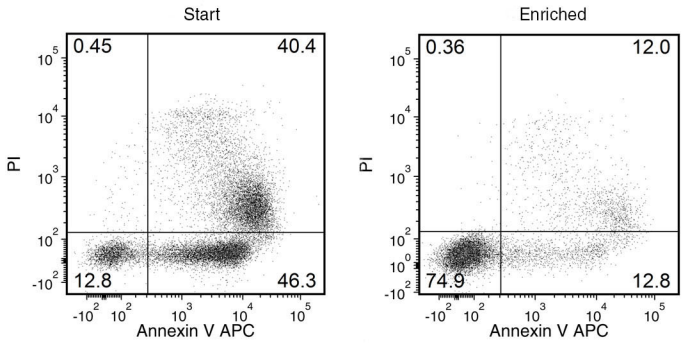
ASSESSING PURITY

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

- Fluorochrome-conjugated Annexin V (Catalog #100-0330), and
- Propidium Iodide (Catalog #75002)

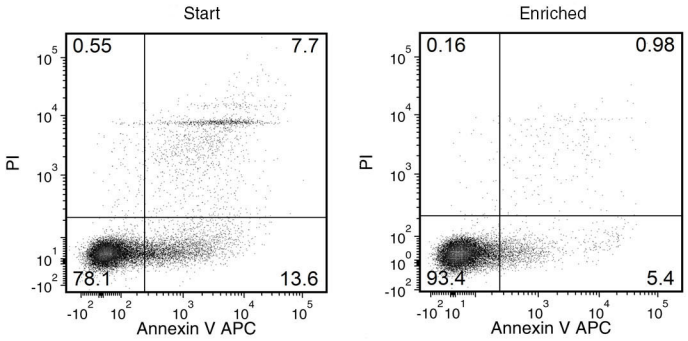
Data

A Human PMNCs with High Percentage of Annexin V+ Cells



Starting with human polymorphonuclear cells (PMNCs) cultured overnight, the live cell content (Annexin V-/PI-) of the enriched fraction is typically 69.7 ± 12.5% (mean ± 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 Annexin V+ Cells



Starting with 24- to 48-hour-old mouse splenocytes, the live cell content of the enriched fraction is typically 79.8 ± 11.4% (mean ± 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.