EasySep™ Release Mouse CD138 Positive Selection Kit

For processing 2 x 10^9 cells

Catalog #100-0601
Catalog #100-1440 RoboSep™

Positive Selection

Document #10000010039 | Version 01

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | 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

Description

Isolate CD138+ cells from single-cell suspensions of mouse splenocytes or bone marrow by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Up to 95% purity of plasma cells from immunized mouse samples
- No-wash removal of EasySep™ Releasable RapidSpheres™
- Compatible with hybridoma generation protocols, including electrofusion

This kit targets CD138+ cells with antibody complexes recognizing the CD138 surface marker and EasySep™ Releasable RapidSpheres™. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are poured off, while desired cells remain in the tube. Then, bound magnetic particles are removed from the EasySep™-isolated CD138+ cells, which are immediately available for downstream applications such as flow cytometry, cell culture, or hybridoma generation.

Following cell isolation with this EasySep™ Release kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Release Mouse CD138 Positive Selection Cocktail	300-0240	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 with 0.1% rHA.
EasySep™ Mouse FcR Blocker	18731	2 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, 0.1% BSA, and < 0.1% sodium azide.
EasySep™ Isolation Cocktail Enhancer*‡	17900	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A solution that enhances the performance of the isolation cocktail.
EasySep™ Releasable RapidSpheres™ 50201	50201	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.
EasySep™ Release Buffer‡ (Concentrate)	20165	3 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A buffer for release of Releasable RapidSpheres™ from cells following positive selection.

BSA - bovine serum albumin; PBS - phosphate-buffered saline; rHA - recombinant human albumin

*RoboSep™ Release Mouse CD138 Positive Selection Kit (Catalog #100-1440) does not require EasySep™ Isolation Cocktail Enhancer (Catalog #17900) for optimal performance. The use of EasySep™ Isolation Cocktail Enhancer is recommended when performing a manual separation.

‡ When using the "The Big Easy" or EasyEights™ (14 mL tube) EasySep™ Magnets, contact us at techsupport@stemcell.com to request an additional vial of EasySep™ Isolation Cocktail Enhancer and three additional vials of EasySep™ Release Buffer (Concentrate).

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

RoboSep™ Release Mouse CD138 Positive Selection Kit is supplied with EasySep™ EasyTube™-14 (Catalog #20128) for optimal performance. The use of EasySep™ EasyTube™-14 is not required when performing a manual separation.

Sample Preparation

SPLEEN

Disrupt spleen tissue in cold PBS or Hanks' Balanced Salt Solution (HBSS) containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27260). Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in cold recommended medium. Keep cells on ice until ready for use.
NOTE: Use of Spleen Dissociation Medium (Catalog #07915) is not recommended when preparing the cells for separation.

BONE MARROW

Flush bone marrow cells from femur and tibia into cold recommended medium using a syringe equipped with a 23 gauge needle. Disperse aggregates by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes, then resuspend cells at 1 x 10^8 nucleated cells/mL in cold recommended medium. Keep cells on ice until ready for use.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (FBS) with 1 mM EDTA. Medium should be free of Ca++ and Mg++. Keep recommended medium cold until use.

Directions for Use – Manual EasySep™ Protocols

See page 2 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™ Release Mouse CD138 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X). Keep cold until use.	Dilute 1 in 40 with recommended medium. Note: Release buffer (1X) must be prepared on the day of use. Refer to steps 14 and 17 for required volume.	Dilute 1 in 40 with recommended medium. Note: Release buffer (1X) must be prepared on the day of use. Refer to steps 14 and 17 for required volume.
2	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.125 - 2 mL	1 x 10^8 cells/mL 0.125 - 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)
3	Add Mouse FcR blocker to sample and mix.	50 µL/mL of sample	50 µL/mL of sample
4	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
5	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add Releasable RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
7	Add cold 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 5 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
9	Add cold 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
Continue to step 10, next page		Continue to step 10, next page	Continue to step 10, next page

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		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 **EasySep™ (Catalog #18000)**	**"The Big Easy" ‡ (Catalog #18001)** 
10	Add Cocktail Enhancer to sample.	• For spleen samples: 25 µL • For bone marrow samples: 50 µL	For spleen samples: • 50 µL for samples < 4 mL • 100 µL for samples ≥ 4 mL For bone marrow samples: • 100 µL for samples < 4 mL • 200 µL for samples ≥ 4 mL
	Mix by gently pipetting up and down 2 - 3 times and incubate.	On ice for 3 minutes	On ice for 3 minutes
11	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 5 minutes
12	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
13	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 4 x 3-minute separations)	Steps 7 and 8, two more times (total of 4 x 5-minute separations)
14	Add cold release buffer (1X) 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Incubate.	On ice for 3 minutes	On ice for 3 minutes
15	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 5 minutes
16	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pour the supernatant into a new tube and set aside.	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the poured-off fraction in step 19	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the poured-off fraction in step 19
17	Remove the tube from the magnet and add cold release buffer (1X) 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Incubate.	On ice for 3 minutes	On ice for 3 minutes
18	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 5 minutes
19	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 16 Isolated cells are ready for use	• Use a new 14 mL tube for start samples < 4 mL • Use a new 50 mL tube for start samples ≥ 4 mL Combine with poured-off fraction from step 16 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.

‡ When using the "The Big Easy" or EasyEights™ (14 mL tube) EasySep™ Magnets, contact us at techsupport@stemcell.com to request an additional vial of EasySep™ Isolation Cocktail Enhancer and three additional vials of EasySep™ Release Buffer (Concentrate).

Table 2. EasySep™ Release Mouse CD138 Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103) ‡	
		5 mL tube	14 mL tube
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X). Keep cold until use.	Dilute 1 in 40 with cold recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to steps 15 and 18 for required volume.	Dilute 1 in 40 with cold recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to steps 15 and 18 for required volume.
2	Prepare sample at the indicated cell concentration within the volume range and keep cold until use.	1 x 10^8 cells/mL 0.25 - 2 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)
3	Add Mouse FcR blocker to sample and mix.	50 µL/mL of sample	50 µL/mL of sample
4	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
5	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add Releasable RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	On ice for 5 minutes	On ice for 5 minutes
7	Add cold 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
8	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant
9	Add cold 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
Continue to step 10, next page		Continue to step 10, next page	Continue to step 10, next page

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103) ‡	
		5 mL tube	14 mL tube
10	Add Cocktail Enhancer to sample.	• For spleen samples: 25 µL • For bone marrow samples: 50 µL	For spleen samples: • 50 µL for samples < 4 mL • 100 µL for samples ≥ 4 mL For bone marrow samples: • 100 µL for samples < 4 mL • 200 µL for samples ≥ 4 mL
	Mix by gently pipetting up and down 2 - 3 times and incubate.	On ice for 3 minutes	On ice for 3 minutes
11	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes
12	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant
13	Add cold 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes
14	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant
15	Add cold release buffer (1X) 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Incubate.	On ice for 3 minutes	On ice for 3 minutes
16	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
17	Carefully pipette* (do not pour) off the supernatant into a new tube and set aside.	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the poured-off fraction in step 20	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the poured-off fraction in step 20
18	Remove the tube from the magnet and add cold release buffer (1X) 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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Incubate.	On ice for 3 minutes	On ice for 3 minutes
19	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
20	Carefully pipette* (do not pour) off the isolated cell suspension.	Combine with poured-off fraction from step 17 Isolated cells are ready for use	• Use a new 14 mL tube for start samples < 4 mL • Use a new 50 mL tube for start samples ≥ 4 mL Combine with poured-off fraction from step 17 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).

‡ When using the "The Big Easy" or EasyEights™ (14 mL tube) EasySep™ Magnets, contact us at techsupport@stemcell.com to request an additional vial of EasySep™ Isolation Cocktail Enhancer and three additional vials of EasySep™ Release Buffer (Concentrate).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Release Mouse CD138 Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	**Prepare sample at the indicated cell concentration within the volume range.**	1 x 10^8 cells/mL 0.5 - 8 mL
2	**Add sample to required tube.**	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
3	**Add Mouse FcR blocker to sample and mix.**	50 µL/mL of sample
4	**Select protocol.**	• Mouse CD138 Release Positive Selection 100-0601 - small volume (< 4 mL) • Mouse CD138 Release Positive Selection 100-0601 - large volume (4 - 8 mL)
5	**Vortex Releasable RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
6	**Load the carousel.**	Follow on-screen prompts NOTE: When prompted to load a separation tube, place EasySep™ EasyTube™-14 into the magnet.
	Start the protocol.	Press the green “Run” button
7	**Unload the carousel when the run is complete.**	Isolated cells are ready for use

Notes and Tips

EASYSEP™ RELEASE BUFFER

EasySep™ Release Buffer (Concentrate) is supplied as a 40X concentrate; release buffer (1X) must be prepared on the day of use. To prepare release buffer (1X), dilute an appropriate volume 1 in 40 with cold recommended medium. Refer to steps 14 and 17 of Table 1 (or steps 15 and 18 of Table 2) for required volume. Keep diluted release buffer cold until use.

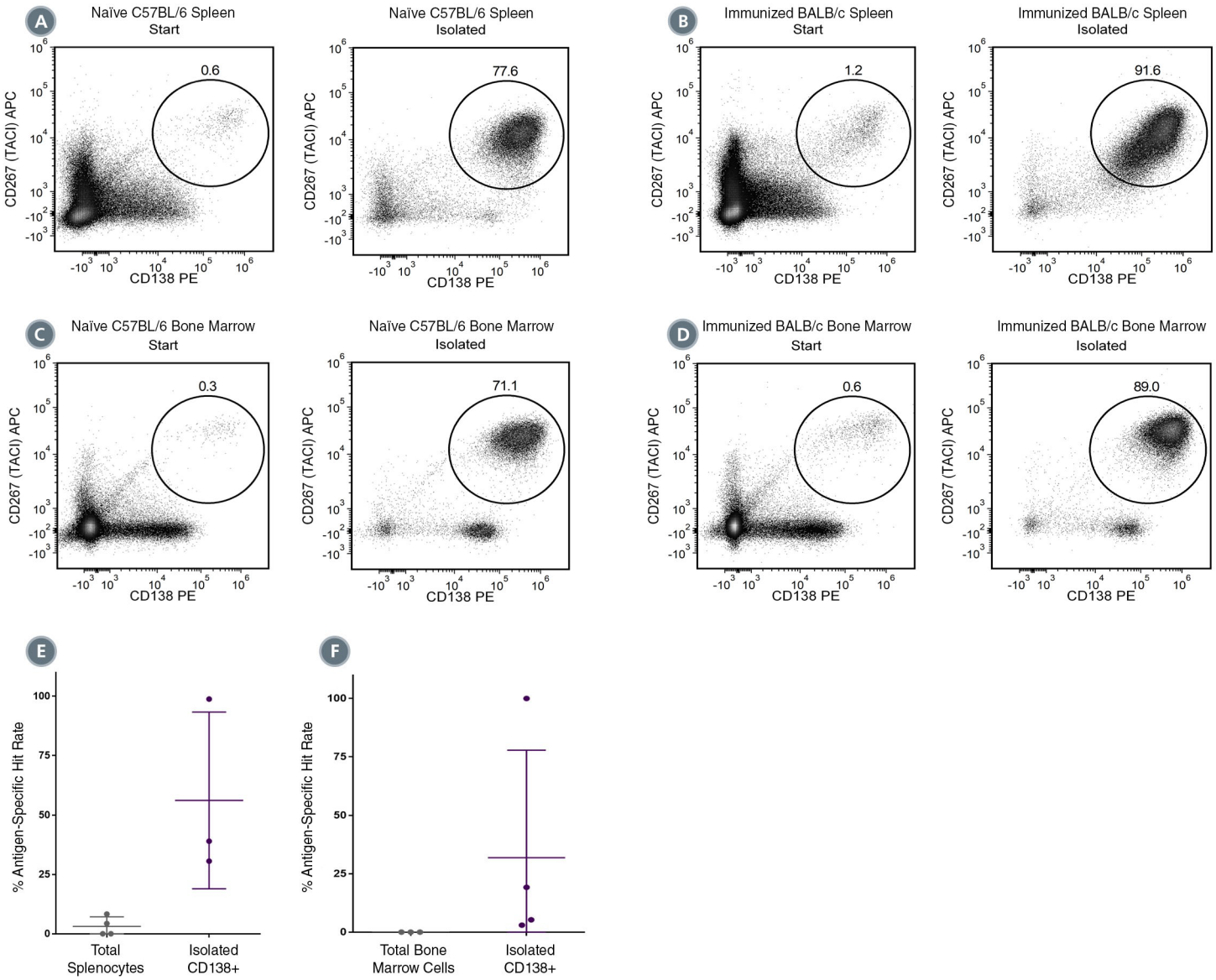
ASSESSING PURITY

For purity assessment by flow cytometry, use the following fluorochrome-conjugated antibody clones:
- Anti-Mouse CD138 (Syndecan-1) Antibody, Clone 281-2 (Catalog #60035)
- Anti-Mouse CD267 (TACI) Antibody, Clone 8F10 (Catalog #60116)
- Anti-Mouse CD45R (B220) Antibody, Clone RA3-6B2 (Catalog #60019)

NOTE: Viability is measured by exclusion of DAPI (Hydrochloride; Catalog #75004), Propidium Iodide (Catalog #75002), or 7-AAD (7-Aminoactinomycin D; Catalog #75001).

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer’s instructions or contact us at techsupport@stemcell.com.

Data



(A) Starting with naïve C57BL/6 mouse splenocytes, the plasma cell (CD138+CD267 (TACI)+) content of the isolated fraction is typically 77.2 ± 2.4% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and isolated fractions are 0.6% and 77.6%, respectively.

(B) Starting with immunized BALB/c mouse splenocytes, the plasma cell (CD138+CD267 (TACI)+) content of the isolated fraction is typically 86.1 ± 7.4% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and isolated fractions are 1.2% and 91.6%, respectively.

(C) Starting with naïve C57BL/6 mouse bone marrow, the plasma cell (CD138+CD267 (TACI)+) content of the isolated fraction is typically 62.0 ± 13.1% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and isolated fractions are 0.3% and 71.1%, respectively.

(D) Starting with immunized BALB/c mouse bone marrow, the plasma cell (CD138+CD267 (TACI)+) content of the isolated fraction is typically 85.5 ± 9.8% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and isolated fractions are 0.6% and 89.0%, respectively.

(E) Isolated CD138+ cells or total splenocytes from mice immunized with various antigens were fused with Sp2/0 mouse myeloma cells and plated in semi-solid medium using ClonaCell™-HY Hybridoma Kit (Catalog #03800). The % antigen-specific hit rate was determined by ELISA. The % antigen-specific hit rates for total splenocytes and CD138+ cells were 3.2 ± 4.0% and 56.1.8 ± 37.2% (mean ± SD), respectively.

(F) Isolated CD138+ cells or total bone marrow cells from mice immunized with various antigens were fused with Sp2/0 mouse myeloma cells and plated in semi-solid medium using ClonaCell™-HY Hybridoma Kit (Catalog #03800). The % antigen-specific hit rate was determined by ELISA. The % antigen-specific hit rates for total bone marrow cells and CD138+ cells were 0% and 31.9 ± 46.0% (mean ± SD), respectively.