EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II

For processing 60 mL whole blood or bone marrow

Catalog #17887

Positive Selection

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STEMCELL™ TECHNOLOGIES

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Description

Isolate highly purified CD138+ (syndecan-1) cells from fresh bone marrow or whole blood by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns required

This kit targets CD138+ cells for positive selection with an antibody recognizing the CD138 surface marker. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as fluorescence in situ hybridization (FISH), flow cytometry, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II Cocktail	17887C	3 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 0.09% rHA. Includes an Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50100	50100	3 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™ Red Blood Cell Lysis Buffer, 10X Concentrate	20110	1 x 10 mL	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.	A 10X concentrated red blood cell lysis reagent.

PBS - phosphate-buffered saline; rHA - recombinant human albumin

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

Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
EasySep™ Red Blood Cell Lysis Buffer (1X dilution)	Store at 2 - 8°C. Do not freeze.	Stable for up to 3 months. Do not exceed the expiry date (EXP) of the original component.

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

Collect whole blood in a blood collection tube containing anticoagulant.

BONE MARROW

To avoid sample degradation and the loss of CD138 from the fragile plasma cells, the samples should be processed as soon as possible and within 72 hours after collection.

1. Dilute the sample 5- to 10-fold in D-PBS (Without Ca++ and Mg++; Catalog #37350) and mix gently by pipetting up and down.
2. OPTIONAL (recommended): Pre-wet a 70 µm strainer with D-PBS. Filter the sample through the pre-wetted strainer to remove bone fragments, cell aggregates, and debris. Rinse the strainer with D-PBS.
3. Centrifuge cells at 300 x g for 10 minutes with the brake off.
4. Using a pipette, carefully remove and discard the plasma, without disturbing the cell pellet. Do not pour.

   OPTIONAL: For bone marrow samples > 24 hours old, add DNase I Solution (1 mg/mL; Catalog #07900) at 100 µg/mL of the original sample volume to help reduce cell clumping. DNase I Solution can be added directly to the pelleted cells with gentle mixing. Incubate at room temperature (15 - 25˚C) for 15 - 30 minutes prior to beginning the EasySep™ protocol.

   NOTE: Avoid repeated freeze-thaw cycles of DNase I Solution.
5. Resuspend the cell pellet with EasySep™ Buffer:
   - If the sample has low cellularity, or if the sample volume is ≥ 2.5 mL, resuspend to the original sample volume.
   - If the sample volume is < 2.5 mL and has high cellularity or cellularity is unknown, resuspend to twice the original sample volume.

Recommended Medium

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

Directions for Use – Manual EasySep™ Protocols

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

Table 1. EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	"The Big Easy" EasySep™ Magnet (Catalog #18001) 
1	Prepare sample within the volume range.	0.5 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 3 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add RapidSpheres™ to sample.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 3 minutes
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 5 mL for diluted samples < 2.5 mL • Top up to 10 mL for diluted samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes
7	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
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 5 mL for diluted samples < 2.5 mL • Top up to 10 mL for diluted samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes
9	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
10	Repeat steps as indicated.	Steps 8 and 9 (total of 1 x 10-minute and 2 x 3-minute separations)
11	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	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™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	 **EasyEights™ (Catalog #18103)** **14 mL tube**
1	Prepare sample within the volume range.	0.5 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 3 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add RapidSpheres™ to sample.	25 µL/mL of diluted sample
	Mix and incubate.	RT for 3 minutes
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 5 mL for diluted samples < 2.5 mL • Top up to 10 mL for diluted samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes
7	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant
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 5 mL for diluted samples < 2.5 mL • Top up to 10 mL for diluted samples ≥ 2.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes
9	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant
10	Repeat steps as indicated.	Steps 8 and 9 (total of 1 x 10-minute and 2 x 5-minute separations)
11	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	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™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	**Prepare sample within the volume range.**	0.5 - 4.5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add 1X EasySep™ RBC Lysis Buffer to sample.**	Equal volume to sample
3	**Select protocol.** **NOTE: Enter volume.**	Human CD138 WB and BM Positive Selection II 17887 NOTE: Enter diluted sample volume.
4	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
5	**Load the carousel.** **NOTE: Do not vortex cocktail.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
6	**Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.**	Isolated cells are ready for use

Notes and Tips

EASYSEP™ RED BLOOD CELL LYSIS BUFFER

EasySep™ Red Blood Cell Lysis Buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

*Type I water refers to ultrapure water suitable for use in analytical procedures. It is defined by the American Society for Testing and Materials (ASTM) as having a resistivity of > 18 MΩ-cm, a conductivity of < 0.056 µS/cm, and < 50 ppb of total organic carbons (TOC).

ASSESSING PURITY

For purity assessment of CD138+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clone:

- Anti-Human CD138 (Syndecan-1) Antibody, Clone MI15 (Catalog #60003)

One of the following methods can also be used:

- Stain for intracellular κ (kappa) and λ (lambda) light chains (e.g. procedure described by Ahmann et al.). Plasma cells express either the kappa or lambda light chain.
- Use alternative markers such as fluorochrome-conjugated Anti-Human CD38 Antibody, Clone HIT2 (Catalog #60014) and Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018) to detect CD38+CD45 variable cells (Kumar et al.).
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

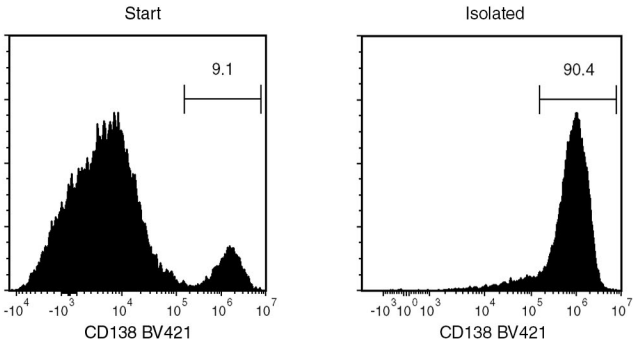
DONOR VARIABILITY

Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic particles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots during flow cytometry analysis of the enriched fraction. This population consists solely of particles, with no cells or platelets present, as determined by staining with fluorescently labeled antibodies against dextran, CD41, and CD45.

When processing whole blood, potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 5- to 10-fold in the recommended medium, and centrifuge at 300 x g for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend the sample to the original volume with recommended medium before beginning the separation procedure.

If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

Data



Starting with fresh whole blood spiked with a multiple myeloma cell line, U266, the CD138+ cell content of the selected fraction typically ranges from 83.7 - 98.3%. In the above example, the purities of the start and final isolated fractions are 9.1% and 90.4%, respectively.

NOTE: For samples with CD138+ starting frequency < 10 - 15%, the CD138+ purity of the isolated fraction may be variable.

NOTE: Red blood cells were removed from the start sample by lysis prior to flow cytometry.

References

Ahmann GJ et al. (1998) A novel three-color, clone-specific fluorescence in situ hybridization procedure for monoclonal gammopathies. Cancer Genet Cytogenet 101(1): 7–11.

Kumar S et al. (2010) Immunophenotyping in multiple myeloma and related plasma cell disorders. Best Pract Res Clin Haematol 23(3): 433–51.