EasySep™ Human Bone Marrow CD138 Positive Selection Kit

For processing up to 60 mL bone marrow

REF 100-0748
Positive Selection

Document #10000013049 | Version 02

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For *In Vitro* Diagnostic Use

℞ Only

Intended Use

EasySep™ Human Bone Marrow CD138 Positive Selection Kit is an in vitro diagnostic device intended to enrich CD138+ cells from bone marrow collected from patients diagnosed with multiple myeloma. The CD138+ cells are enriched by immunomagnetic positive selection for use in validated downstream assays. The end-user is responsible for validation of this kit for use with the assay. For in vitro diagnostic use by laboratory professionals.

Background

Multiple myeloma is a hematological malignancy characterized by the accumulation of plasma cell neoplasms in the bone marrow.[1] In the United States, multiple myeloma accounts for 1.8% of all cancers and is typically diagnosed among people aged 65 to 74 years.[2] Several subtypes of disease have been identified at the genetic and molecular level, and specific chromosomal abnormalities have prognostic implications that enable the risk stratification of patients.[2]

Consensus recommendations established by US and EU oncology experts and captured in guidelines published by the National Comprehensive Cancer Network (NCCN)[2], the European Society for Myeloma[3], and the European Myeloma Network[4] recommend cytogenetic analysis of plasma cells obtained from bone marrow specimens by FISH at initial diagnosis and as a follow-up and surveillance method during relapse.[2-4]

The frequency of plasma cells in bone marrow from multiple myeloma patients can be highly variable at diagnosis and during relapse. Plasma cells can be isolated from bone marrow by the immunomagnetic positive selection of cells expressing the CD138 (syndecan-1) surface antigen.[5] The enrichment of plasma cells from bone marrow prior to FISH testing for multiple myeloma has been reported to increase the rate and frequency of genetic abnormalities detected by FISH (Figure 1).[5-7]

Principle of Operation

EasySep™ Human Bone Marrow CD138 Positive Selection Kit is designed to be used manually to enrich cells expressing the CD138 surface antigen from fresh human whole bone marrow samples by immunomagnetic positive selection. 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 using a "The Big Easy" EasySep™ magnet. Unwanted cells are poured off, while isolated CD138+ cells remain in the tube.

Component Descriptions

EasySep™ Human Bone Marrow CD138 Positive Selection Kit consists of the following components listed under Table 1.

Table 1. EasySep™ Human Bone Marrow CD138 Positive Selection Kit Components

Component Name	Component #	Quantity	Content
EasySep™ Human Bone Marrow CD138 Positive Selection Cocktail	300-0394	3 x 1 mL*	A combination of monoclonal antibodies in D-PBS with 0.09% rHA. Includes an Fc receptor blocking antibody.
EasySep™ Dextran RapidSpheres™ 50105	300-0400	3 x 1 mL*	A suspension of magnetic particles in water.

* 1 mL vial of each component can be used for processing up to 20 mL of bone marrow depending on the level of cellularity of the sample (refer to Directions for Use, Step 5 of this document).
D-PBS - Dulbecco's phosphate-buffered saline; rHA - recombinant human albumin

Material Provided

EASYSEP™ RED BLOOD CELL LYSIS BUFFER

EasySep™ Human Bone Marrow CD138 Positive Selection Kit is provided with EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate (Catalog #100-0749), 1 x 20 mL. This 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.

Refer to the EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate (Catalog #100-0749) Product Information Sheet (PIS; Document #10000017274), available at www.stemcell.com/0749. Contact the toll free number provided in the Technical Assistance section of this document to request a paper copy of this PIS free of charge.

*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).

Quality Control

EasySep™ Human Bone Marrow CD138 Positive Selection Kit is manufactured in compliance with 21 CFR Part 820 and ISO 13485. For additional quality information, refer to www.stemcell.com/compliance.

Storage and Stability

Table 2. Storage and Stability

Component Name	Component #	Storage
EasySep™ Human Bone Marrow CD138 Positive Selection Cocktail	300-0394	2 - 8°C
EasySep™ Dextran RapidSpheres™ 50105	300-0400	2 - 8°C
EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate	100-0749	room temperature (RT; 15 - 25°C)

Components may be shipped at RT but should be stored as indicated above. Do not freeze. All components are stable until use-by date on the label.

NOTE: EasySep™ Red Blood Cell Lysis Buffer (1X) can be stored at 2 - 8°C for up to 3 months. Do not exceed the expiry date (use-by date) of the original 10X buffer.

Accessories Required but Not Provided

Materials listed in Table 3 are accessories to EasySep™ Human Bone Marrow CD138 Positive Selection Kit. These materials are not provided with the kit and can be purchased separately.

Table 3. Accessories Required but Not Provided

Component Name	Component #	Quantity	Storage	Shelf Life	Description
"The Big Easy" EasySep™ Magnet	18001	1	Store at 15 - 25°C.	Not applicable	A magnet for column-free immunomagnetic separation.
EasySep™ Buffer	100-0780	1 x 1 L	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.	Cell separation buffer.

"THE BIG EASY" EASYSEP™ MAGNET

"The Big Easy" EasySep™ Magnet generates a high gradient magnetic field for cell separation and is designed for use with EasySep™ Human Bone Marrow CD138 Positive Selection Kit. "The Big Easy" EasySep™ Magnet is designed to hold a standard 14 mL (17 x 95 mm) polystyrene round-bottom tube.

EASYSEP™ BUFFER

Refer to the EasySep™ Buffer (Catalog #100-0780) PIS (Document #10000016106), available at www.stemcell.com/0780. Contact the toll-free number provided in the Technical Assistance section of this document to request a paper copy of this PIS free of charge.

Materials Needed but Not Provided

Materials listed below are general purpose laboratory items that are required but not provided.

- Dulbecco's phosphate-buffered saline (without Ca++ and Mg++; e.g. Catalog #37350)
- DNase I solution (e.g. Catalog #07900)
- 70 µm strainer (e.g. Catalog #27216)
- 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
- 50 mL conical tube (e.g. Catalog #38010)

Limitations

1. FOR IN VITRO DIAGNOSTIC USE
2. The use of the device is intended for the enrichment of CD138+ cells from patients that are known to have or may have a cancer diagnosis.
3. The performance characteristics of this device have not been established for general downstream diagnostic assays. End users need to validate use with any subsequent tests and specimen collection devices.
4. The standalone device is not intended for cell enumeration. The device is intended only for enriching CD138+ cells in specimens so that the enriched specimens can then be used in further processing/analysis using additional independent methods, such as FISH.
5. The standalone device is not intended for diagnostic, prognostic, or monitoring use with CD138+ cells, including as an aid in any disease management and/or treatment decisions. Results from the standalone device do not provide information to the patient regarding their current state of health. The standalone device does not diagnose any health conditions and is not a substitute for visits to a doctor or other healthcare professional.
6. Resuspend the bone marrow specimen based on specimen volume or cellularity levels as per the instructions provided in Sample Preparation.
7. Follow all protocol steps listed in Directions for Use. Improper execution of protocol may lead to variable and/or poor results.
8. A low CD138+ (< 3%) starting frequency may lead to variability in purity of enriched CD138+ cells. End users may assess the purity of CD138+ cells after enrichment by either immunophenotyping using flow cytometry or morphological assessment by microscopy.
9. Bone marrow stability may be compromised after 48 hours from collection.
10. The number of CD138+ plasma cells isolated from bone marrow by immunomagnetic selection may be reduced in patients undergoing or following treatment for multiple myeloma.
11. If no abnormal FISH patterns are observed when multiple FISH probes are used, seek technical assistance by contacting techsupport@stemcell.com.

Warnings and Precautions

1. Important information regarding the safe handling, transport, and disposal of these products is contained in the Safety Data Sheets (SDS).

   NOTE: SDS for the reagents provided in the kit are available at www.stemcell.com.
2. This product should be handled by trained personnel observing good laboratory practices. Once this product is added to human cells, treat the suspension as potentially biohazardous. Handling of reagents and disposal of wastes should observe all local, state, or national regulations.
3. This product is a potential irritant to eyes, respiratory system, and skin. This product may also be harmful if ingested. Avoid exposure through skin, eye contact, inhalation, and ingestion.
4. CAUTION: EasySep™ Magnet generates a strong magnetic field. Keep away from pacemakers, magnets, computer disks, watches, and other objects that respond to magnetic fields.
5. Ensure that vials are capped properly during storage. Proper handling and storage of kit components is essential to ensure the labeled shelf life and performance of the kit. CD138+ cell enrichment may be adversely affected by kit components stored outside of the recommended storage conditions.
6. Do not use the product if packaging container's integrity has been compromised upon arrival or if there are any signs of contamination, drying, leaks, or any other evidence of deterioration. Unused product may be disposed of according to standard laboratory procedures for non-hazardous liquids.
7. Do not use the product beyond the use-by date indicated on its label.
8. Do not freeze EasySep™ Dextran RapidSpheres™ 50105. If EasySep™ Dextran RapidSpheres™ 50105 are clumped or cannot be evenly dispersed, contact techsupport@stemcell.com.
9. Do not vortex EasySep™ Human Bone Marrow CD138 Positive Selection Cocktail.
10. EasySep™ Human Bone Marrow CD138 Positive Selection Kit is designed for use with a minimum of 1 mL of fresh bone marrow. Samples should be processed as soon as possible, preferably within 24 hours but no longer than 72 hours after bone marrow aspirate collection. Do not freeze or transport bone marrow in direct contact with cold/ice packs or expose to temperatures above 30°C prior to use. Follow the bone marrow sample preparation instructions provided in Sample Preparation.

Directions for Use

SAMPLE PREPARATION

EasySep™ Human Bone Marrow CD138 Positive Selection Kit is designed for use with fresh human whole bone marrow. Recommendations for specimen collection can be found in the AGT Cytogenetics Laboratory Manual.[8] Bone marrow aspirate collections should be performed according to the laboratory's institutional guidelines. At least 1 mL of bone marrow aspirate should be collected in a blood collection tube containing an anticoagulant.

To avoid sample degradation, loss of CD138 from the fragile plasma cells, and to maintain cell viability, the samples should be processed as soon as possible, preferably within 24 hours but no longer than 72 hours after bone marrow aspirate collection. Do not freeze or transport bone marrow in direct contact with cold/ice packs, or expose to temperatures above 30°C prior to use.

Optimal performance of the protocol requries appropriate specimen collection, storage, and transport to the test site.

Follow steps listed below to prepare the sample for cell enrichment:

1. Dilute bone marrow 5- to 10-fold in Dulbecco's phosphate-buffered saline (D-PBS) and mix gently by pipetting up and down.
2. 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 the cells at 300 x g for 10 minutes (acceptable range of 250 - 300 x g for 8 - 12 minutes) with the brake off.
4. Using a pipette, carefully remove and discard the plasma, without disturbing the buffy coat/red cell pellet. Do not pour. When working with bone marrow samples that are > 24 hours old, add 50 µL of 1 mg/mL DNase I solution directly to the buffy coat/red blood cell pellet and resuspend. Incubate at room temperature (15 - 25˚C) for 15 - 30 minutes prior to performing the cell separation.

   NOTE: Store DNAse I solution at -20°C. Aliquot DNase I solution into working volumes to avoid repeated freeze-thaw cycles.

   Tip: Alternatively, DNAse I solution stored at 2 - 8°C can be used for up to 1 week.
5. Resuspend the buffy coat/red blood cell pellet to 1 - 2X the original starting bone marrow sample volume with EasySep™ Buffer (Table 4).
   - If original sample volume was ≥ 2.5 mL, dilute to the original sample volume.
   - If original sample volume was < 2.5 mL, dilute to twice the original sample volume.
   - Alternatively, if the sample is expected to have high cellularity (> 70%) or cellularity is unknown, dilute to twice the original sample volume.

Table 4. EasySep™ Buffer Volumes

Original Sample Volume	Sample Volume After Resuspension with EasySep™ Buffer
1 mL	2 mL
2 mL	4 mL
2.5 mL	2.5 mL
3 mL	3 mL
4 mL	4 mL
4.5 mL	4.5 mL

CELL SEPARATION

6. Prepare EasySep™ Red Blood Cell Lysis Buffer (referred to as EasySep™ RBC Lysis Buffer) (1X) at least 1 hour before use. EasySep™ RBC Lysis Buffer is supplied as a 10X concentrate.
   a. Add 1 part EasySep™ RBC Lysis Buffer to 9 parts distilled or Type 1 water.
   b. Mix gently and completely before use.

   NOTE: If not used immediately, store EasySep™ RBC Lysis Buffer (1X) at 2 - 8°C for up to 3 months. Do not freeze.
7. Prepare the sample within the volume range of 1 - 4.5 mL and add sample to required tube. If the volume is > 4.5 mL, split the sample between two 14 mL round-bottom tubes.
8. Add an equal volume of EasySep™ RBC Lysis Buffer (1X) prepared in step 6 to the sample. For example, if the sample volume is 2 mL after resuspension with EasySep™ Buffer (step 5), add 2 mL of EasySep™ RBC Lysis Buffer (1X) to the sample.
9. Add 25 µL of EasySep™ Human Bone Marrow CD138 Positive Selection Cocktail per 1 mL of diluted sample (step 8). Mix and incubate at room temperature (15 - 25˚C) for 3 minutes (up to a maximum of 5 minutes). For example, if diluted sample volume is 4 mL, add 100 µL of EasySep™ Human Bone Marrow CD138 Positive Selection Cocktail to sample.

   NOTE: Do not vortex cocktail.
10. Vortex EasySep™ Dextran RapidSpheres™ 50105 for at least 30 - 40 seconds. Particles should appear evenly dispersed.
11. Add 25 µL of EasySep™ Dextran RapidSpheres™ 50105 per 1 mL of diluted sample. Mix and incubate at room temperature (15 - 25˚C) for 3 minutes (up to a maximum of 5 minutes).
12. Add EasySep™ Buffer to top up the sample to the volume indicated below. 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

13. Place the tube (without lid) into "The Big Easy" EasySep™ Magnet and incubate at room temperature (15 - 25˚C) for 10 minutes (up to a maximum of 11 minutes).
14. Pick up the magnet and, in one continuous motion, invert the magnet and tube to pour off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells. Discard the supernatant.

    NOTE: 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.
15. Add EasySep™ Buffer to top up the sample to the volume indicated below. 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
16. Place the tube (without the lid) into the magnet and incubate at room temperature (15 - 25˚C) for 3 minutes (up to a maximum of 5 minutes).
17. Pick up the magnet and, in one continuous motion, invert the magnet and tube to pour off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells. Discard the supernatant.

    NOTE: 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.
18. Add EasySep™ Buffer to top up the sample to the volume indicated below. 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
19. Place the tube (without the lid) into the magnet and incubate at room temperature (15 - 25˚C) for 3 minutes (up to a maximum of 5 minutes).
20. Pick up the magnet and, in one continuous motion, invert the magnet and tube to pour off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells. Discard the supernatant.

    NOTE: 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.
21. Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube. Enriched cells can be used in further processing/analysis using additional independent methods, such as FISH assay, that have been validated by end users.

Performance Summary

CLINICAL STUDY

A clinical study was conducted using the EasySep™ Human Bone Marrow CD138 Positive Selection Kit on 33 clinical bone marrow specimens from multiple myeloma patients at various stages of disease. The clinical specimens were evaluated using a panel of five common FISH probes detecting six genomic abnormalities that included a CCND1/IGH XT probe to detect the t(11;14) translocation, as well as probes to detect chromosome 5 (D5S23) and chromosome 15 polysomy (D15Z4) (single probe kit), MYC breakapart, 13q deletion (D13S319), and TP53 deletion (D17Z1). Of the 33 patients, 32 were positive for at least one FISH probe, and 14 patients had at least one FISH probe that gave an abnormal signal pattern only after EasySep™ CD138 enrichment. CD138 purity, as measured by flow cytometry, was 12.9% (range 0.2% - 82.7%) in unenriched specimens and increased to 79.6% (range 18.5% - 98.6%) in the EasySep™ CD138-enriched specimens (Table 5). Fold enrichment for CD138 purity was > 1 and varied based on initial frequency* for all specimens with CD138 frequencies < 40% prior to enrichment. In this study, fold enrichment was 65.9 (range 29.6 - 91.5) for specimens with initial CD138 frequencies < 3%, 11.9 (range 5.1 - 23.0) for specimens with initial CD138 frequencies between 3 - 15%, and 3.2 (1.6 - 5.3) for specimens with initial CD138 frequencies between 15 - 40% prior to enrichment.

*NOTE: For specimens with CD138 frequencies > 40%, plasma cell enrichment prior to FISH may not be required.[9]

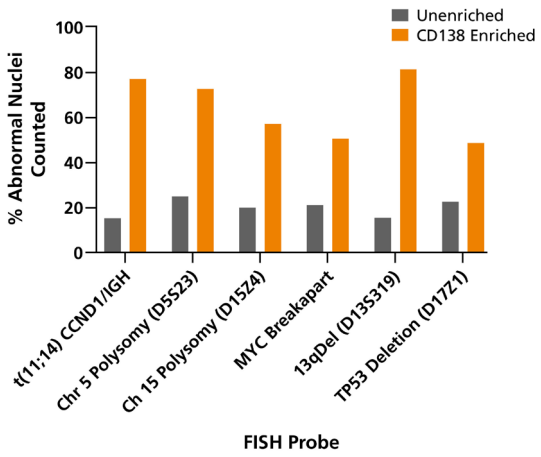
Table 5. CD138 Purity and Percentage of Abnormal Nuclei Detected with Five FISH Probes Pre- and Post-EasySep™ CD138 Enrichment for 33 Multiple Myeloma Bone Marrow Aspirates

Multiple Myeloma Patient Sample	CD138 Frequency*	Pre-Enriched CD138 Purity	Enriched CD138 Purity	Fold Enrich-ment[&]	% Abnormal Nuclei											
					Probe (D5S23)		Probe (D15Z4)		Probe (MYC)		Probe (D13S319, 13qter)		Probe (IGH/ CCND1 XT)		Probe (TP53, D17Z1)	
					Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre- Enr	Enr
1	medium	5.0	57.3	10.5	0	0	0	0	0	0	0	51	13	53	0	0
2	high	15.5	91.4	4.9	35.5	87	21.5	63	0	0	0	0	0	0	0	0
3	high	15.6	98.2	5.3	0	0	0	0	0	17	19	88	0	0	0	0
4	high	82.7	77.7	-0.1	38	45	34.5	30	69	91	50.5	74	0	0	54	70
5	medium	4.3	57.0	12.3	25.5	74	28.5	67	0	0	0	0	0	8.5	0	0
6	medium	6.5	98.6	14.2	0	0	19	44	25.5	46	14.5	73	32.5	92	26.5	77
7	medium	10.0	94.4	8.4	20	69	17.5	71	0	0	0	0	0	0	0	0
8	high	36.2	93.7	1.6	45	88	0	0	39	53	0	0	54	94	0	0
9	medium	13.8	92.0	5.7	0	0	0	0	29.5	89	24.5	89	0	0	0	0
10	high	31.4	85.7	1.7	0	0	9	75	0	0	0	90	18	95.5	0	0
11	medium	7.9	85.6	9.8	0	0	0	0	0	0	0	95	9.5	91.5	0	0
12	high	16.2	91.0	4.6	29.5	85	16	51	0	0	41	93	21.5	86.5	0	0
13	medium	3.7	88.9	23.0	0	0	0	0	0	0	0	0	0	0	0	0
14	medium	12.4	75.3	5.1	14.5	47	0	0	23.5	88	24	97	0	0	9.5	43
15	medium	7.7	81.7	9.6	0	0	17	53	0	6	20.5	93	0	8.5	0	0
16	high	35.0	95	1.7	0	0	0	0	0	0	40	88	0	0	46.5	81
17	medium	4.6	82.9	17.0	0	0	0	0	0	0	18	77	8	82	0	22
18	low	0.7	64.5	91.1	0	0	0	0	0	0	0	0	4	85.5	0	0
19	medium	4.9	88.3	17.0	10.5	75	7	27	0	0	0	0	0	0	0	0
20	high	27.7	96.8	2.5	77	91	68	81	0	0	0	0	0	0	0	0
21	low	0.2	18.5	91.5	1.5	48	0	0	0	0	0	50	0	0	0	0
22	low	1.7	52.0	29.6	0	0	0	0	0	0	0	80	0	0	0	0
23	high	17.2	NC	n/a	15	95	16	93	0	0	0	0	29	92	0	0
24	medium	5.7	NC	n/a	0	0	0	0	0	0	0	90	0	0	0	0
25	low	2.3	NC	n/a	0	0	0	0	0	0	0	0	7	64	0	0
26	medium	10.6	96.3	8.1	8.5	68	9	73	7.5	37	0	0	0	0	0	0
27	low	1.5	80.6	52.7	0	0	0	4	0	0	0	49	0	77	0	0
28	high	20	92.1	3.6	0	0	4	61	0	0	40	91	0	0	0	0
29	medium	9.7	90.6	8.3	21.5	75	13.5	55	0	8	18.5	84	0	0	0	0
30	low	0.5	32.8	64.6	5	53	0	0	0	0	0	0	0	0	0	0
31	medium	5	84.6	15.9	0	0	0	0	0	0	0	0	4	83	0	0
32	medium	5.8	87.5	14.1	0	0	0	0	0	0	0	80	0	0	0	0
33	medium	4.3	56.2	12.1	28	91	28	87	18	72	0	96	0	0	0	0

* low = < 3% CD138 initial frequency; medium = 3 - 15% CD138 initial frequency; high = > 15% CD138 start frequency
[&] Fold enrichment is calculated using the following formula: [Enriched % CD138 Purity / Pre-enriched % CD138 Purity] - 1
Enr - enriched; Pre-Enr - pre-enriched; NC - not collected

Figure 1 was generated using a subset of the data shown in Table 5 (the complete data set), where only specimens with an abnormal FISH signal pattern for each probe were included in the analysis. Figure 1 shows the percentage of abnormal nuclei detected for each probe was higher following EasySep™ CD138 enrichment compared to the sample before enrichment.

Figure 1. Percentage of Abnormal Nuclei Counted in Plasma Cells Before (Unenriched) and After Enrichment Using EasySep™ Human Bone Marrow CD138 Positive Selection Kit



Only specimens with an abnormal FISH signal pattern for each probe were included in the analysis. The percentage of abnormal nuclei before (unenriched) and after enrichment increased by the following amounts for each probe: CCND1/IGH (t(11;14)) probe: 15.4 ± 15.5% to 77.3 ± 29.5% (n = 14); chromosome 5 (D5S23) probe: 25.0 ± 19.1% to 72.7 ± 17.4% (n = 15); chromosome 15 (D15Z4) probe: 20.0 ± 15.9% to 57.3 ± 23.6% (n = 16); MYC breakapart probe: 21.2 ± 21.7% to 50.7 ± 33.5% (n = 10); 13q deletion (D13S319) probe: 15.5 ± 16.9% to 81.4 ± 15.2% (n = 19); TP53 (D17Z1) probe: 22.8 ± 23.5% to 48.8 ± 32.9% (n = 5).

ANALYTICAL PERFORMANCE STUDIES

REPRODUCIBILITY STUDY

Reproducibility with Contrived Samples

A study was performed to demonstrate reproducibility of the enrichment process across three study sites using three EasySep™ Human Bone Marrow CD138 Positive Selection Kit lots. Due to challenges with having adequate samples for three sites, the study was performed using contrived samples generated by spiking a multiple myeloma cell line into healthy donor whole blood each day at either low (< 3%), medium (3 - 15%), or high (>15%) initial CD138 frequency, as determined by flow cytometry, to generate 16 distinct panel members. More panel members were studied at the low and medium levels (6 versus 4 for the high level) to demonstrate enrichment at the more challenging levels.

Table 6 shows a statistical summary of the overall, site-to-site, lot-to-lot variability, and fold-enrichment from the high, medium, and low CD138+ panel members. Enrichment increased a mean of 85.7-fold in the low CD138+ initial frequency samples, 9.3-fold in the CD138+ medium frequency samples, and 3.8-fold in the high CD138+ initial frequency samples.

Table 6. EasySep™ CD138 Reproducibility Study Variability and Fold Enrichment Summary

CD138+ Initial Frequency	N	Repeatability CV (%)	Between Site CV (%)	Between Lot CV (%)	Reproducibility CV (%)	Fold Enrichment
		Mean (Min. - Max)	Mean (Min. - Max)	Mean (Min. - Max)	Mean (Min. - Max)	Mean (Min. - Max)
High (> 15%)	4	0.7 (0.5 - 0.9)	4.0 (2.8 - 5.9)	0.9 (0.5 - 1.6)	4.0 (2.8 - 6.0)	3.8 (2.6 - 5.5)
Med (3 - 15%)	6	1.6 (0.8 - 3.0)	4.2 (2.1 - 6.8)	2.3 (1.0 - 3.9)	4.5 (2.2. - 7.0)	9.3 (5.0 - 15.4)
Low (< 3%)	6	5.4 (3.5 - 8.9)	9.7 (5.5 - 13.8)	8.6 (4.5 - 14.2)	11.9 (8.2 - 18.3)	85.7 (20.2 - 206.0)

CV - coefficient of variation

Table 7 shows the mean plus minimum and maximum CD138+ purity for unenriched and enriched samples from all panel members across the three study sites. Low CD138+ panel members had mean CD138+ cell purities ranging from 0.7% - 1.5% before enrichment and increased to mean CD138+ cell purities of 56.2% - 79.6% after EasySep™ enrichment. Medium CD138+ panel members had mean CD138+ cell purities ranging from 7.0% - 12.3% before enrichment and increased to mean CD138+ cell purities of 85.5% - 92.6% after EasySep™ enrichment. High CD138+ panel members had mean CD138+ cell purities ranging from 17.9% - 22.8% before enrichment and increased to mean CD138+ cell purities of 88.5% - 94.0% after EasySep™ enrichment.

Table 7. Unenriched and Enriched CD138+ Cell Purity for Low, Medium, and High CD138+ Panel Members as Determined by Flow Cytometry Analysis by All Three Study Sites

Target Frequency	Panel Member	Unenriched Purity (%)			Enriched Purity (%)		
		Mean	Min	Max	Mean	Min	Max
Low	1	1.5	0.8	2	56.6	43.1	74.9
	5	1.3	0.7	2	73	62.3	86.7
	8	0.7	0.6	0.8	56.2	43.8	66.5
	12	0.7	0.4	0.9	76.9	66.4	89.3
	14	1.2	0.6	1.8	79.6	71.4	91.2
	15	0.8	0.4	1.2	74.9	57.9	82.8
Med	2	9.4	7.2	11.3	89.8	79.6	95.3
	3	9.2	7	12.5	88.9	84.3	93
	7	7.3	5.3	9.9	86.3	81.5	90.4
	10	7	5.5	8.3	86.4	75.3	93.2
	13	12.3	11.1	14.3	92.6	89.7	95.4
	16	10.4	6.2	14.6	85.5	78.7	90.4
High	4	19	16.4	21.3	94	89.9	96.8
	6	22.8	19.3	26.6	92.3	87.8	96.4
	9	18	17	18.6	88.5	82.9	95.2
	11	17.9	14.3	20	93.2	90.3	96.3

Separate analyses of variance were performed within each of the three sites and the three kit lots based on flow cytometry before and after enrichment. Table 8 shows analysis results by kit lot and panel member, with precision estimates for repeatability and within lot/between sites. Similarly, Table 9 shows analysis results by site and panel member, with precision estimates for repeatability and within site/between lots. In both sets of analyses, all medium and high CD138+ panel members within site and lot had CV < 15%. Similarly, all low CD138+ panel members within site and lot had CV < 35%, which met the acceptance criteria due to the expected higher variability for low CD138+ initial frequency samples. Thus, the variability is well-controlled within lot (across site) and within site (across lot).

Table 8. Precision Estimates by Kit Lot and Panel Member

Kit Lot	Panel Member	Mean Unenriched Purity*	Mean Enriched Purity**	Repeatability		Within Lot	
				SD	CV (%)	SD	CV (%)
1	8	0.66	60.17	2.13	3.5	5.01	8.3
	12	0.71	72.48	1.21	1.7	8.39	11.6
	15	0.82	68.15	4.93	7.2	6.23	9.1
	14	1.19	77.56	1.71	2.2	4.56	5.9
	5	1.26	75.47	0.38	0.5	9.86	13.1
	1	1.47	55.66	7.13	12.8	7.18	12.9
	10	6.96	87.05	3.4	3.9	5.22	6
	7	7.27	87.26	0.33	0.4	1.17	1.3
	3	9.19	88.02	1.86	2.1	2.42	2.8
	2	9.44	88.91	0.65	0.7	7.47	8.4
	16	10.44	85.49	1.38	1.6	4.93	5.8
	13	12.34	92.23	0.71	0.8	2.1	2.3
	11	17.9	93.32	0.14	0.2	2.49	2.7

Kit Lot	Panel Member	Mean Unenriched Purity*	Mean Enriched Purity**	Repeatability		Within Lot	
				SD	CV (%)	SD	CV (%)
	9	18.01	87.11	1.13	1.3	5.02	5.8
	4	18.99	93.66	0.35	0.4	2.94	3.1
	6	22.84	92	0.45	0.5	3.99	4.3
2	8	0.66	55.67	1.68	3	9.79	17.6
	12	0.71	80.84	1.96	2.4	5.2	6.4
	15	0.82	79.59	1.14	1.4	1.96	2.5
	14	1.19	84.84	2.08	2.4	6.17	7.3
	5	1.26	72.02	2.72	3.8	6.6	9.2
	1	1.47	64.12	2.93	4.6	9.24	14.4
	10	6.96	88.4	2.54	2.9	4.74	5.4
	7	7.27	87.78	0.56	0.6	1.93	2.2
	3	9.19	90.19	0.85	0.9	2.19	2.4
	2	9.44	91.62	0.84	0.9	4.65	5.1
	16	10.44	84.9	0.96	1.1	3.91	4.6
	13	12.34	93.26	1.01	1.1	2.1	2.3
	11	17.9	93.36	0.78	0.8	2.54	2.7
	9	18.01	88.89	0.32	0.4	5.65	6.4
	4	18.99	94.27	0.72	0.8	3.12	3.3
	6	22.84	92.08	0.38	0.4	3.96	4.3
3	8	0.66	52.7	3.68	7	6.54	12.4
	12	0.71	77.45	3.58	4.6	7.67	9.9
	15	0.82	77.05	1.55	2	3.22	4.2
	14	1.19	76.55	2.44	3.2	4.08	5.3
	5	1.26	71.49	1.95	2.7	6.85	9.6
	1	1.47	50.17	1.73	3.5	6.66	13.3
	10	6.96	83.71	1.16	1.4	6.84	8.2
	7	7.27	83.86	0.93	1.1	2.56	3.1
	3	9.19	88.37	0.38	0.4	3.2	3.6
	2	9.44	88.72	0.69	0.8	5.9	6.7
	16	10.44	86.19	1.18	1.4	2.4	2.8
	13	12.34	92.18	0.81	0.9	1.68	1.8
	11	17.9	92.97	0.26	0.3	2.7	2.9
	9	18.01	89.43	0.77	0.9	4.97	5.6
	4	18.99	94.11	0.39	0.4	2.84	3
	6	22.84	92.88	0.86	0.9	2.81	3

*Unenriched purity for the same sample is averaged across differing measurements from the three sites
**Mean enriched purity is the average purity of enriched samples across three study sites

Table 9. Precision Estimates by Site and Panel Members

Site	Panel Member	Unenriched Purity*	Mean Enriched Purity**	Repeatability		Within Site	
				SD	CV (%)	SD	CV (%)
1	8	0.57	60.75	2.69	4.4	3.81	6.3
	12	0.79	84.73	2.98	3.5	3.13	3.7
	15	0.92	76.93	0.72	0.9	2.73	3.5
	14	1.19	76.97	2.55	3.3	3.5	4.5
	5	0.65	81.18	1.08	1.3	4.6	5.7
	1	1.58	64.33	6.66	10.4	10.24	15.9
	10	7.11	87.67	0.43	0.5	2.2	2.5
	7	6.59	85.89	0.71	0.8	2.2	2.6
	3	8.06	89.44	0.94	1.1	1.6	1.8
	2	9.83	93.35	0.49	0.5	0.86	0.9
	16	10.52	85.13	0.59	0.7	1.12	1.3
	13	11.13	92.7	0.47	0.5	0.62	0.7
	11	19.39	92.4	0.14	0.1	0.35	0.4
	9	17.03	86.78	0.9	1	1.22	1.4
	4	19.26	96.03	0.34	0.4	0.48	0.5
	6	22.61	92.49	0.35	0.4	0.73	0.8
2	8	0.6	50.2	2.97	5.9	5.51	11
	12	0.4	73.25	2.41	3.3	6.6	9
	15	0.4	75.6	1.2	1.6	7.93	10.5
	14	0.6	77.93	0.88	1.1	6.25	8
	5	1.1	71.23	0.85	1.2	2.31	3.2
	1	0.8	54.1	2.59	4.8	5.16	9.5
	10	5.5	80.72	3.9	4.8	4.91	6.1
	7	5.3	84.77	0.26	0.3	2.77	3.3
	3	7	86.23	0.52	0.6	1.65	1.9
	2	7.2	82.85	0.78	0.9	3.15	3.8
	16	6.2	82.32	1.85	2.3	3.08	3.7
	13	11.6	90.63	1.23	1.4	1.23	1.4
	11	14.3	91.18	0.8	0.9	0.8	0.9
	9	18.6	84.37	0.31	0.4	1.37	1.6
	4	16.4	90.63	0.82	0.9	0.82	0.9
	6	19.3	88.68	0.89	1	1.27	1.4
3	8	0.82	57.58	2.19	3.8	8.56	14.9
	12	0.94	72.78	1.86	2.6	4.31	5.9
	15	1.15	72.25	5.1	7.1	8.58	11.9
	14	1.79	84.05	2.43	2.9	6.43	7.7
	5	2.04	66.57	3.08	4.6	3.08	4.6
	1	2.03	51.52	3.38	6.6	7.76	15.1
	10	8.28	90.77	2	2.2	2.36	2.6
	7	9.93	88.23	0.85	1	1.83	2.1
	3	12.5	90.9	1.78	2	1.78	2

Site	Panel Member	Unenriched Purity*	Mean Enriched Purity**	Repeatability		Within Site	
				SD	CV (%)	SD	CV (%)
	2	11.3	93.05	0.87	0.9	1.53	1.6
	16	14.6	89.13	0.67	0.7	0.78	0.9
	13	14.3	94.33	0.67	0.7	0.93	1
	11	20	96.08	0.2	0.2	0.2	0.2
	9	18.4	94.28	1.03	1.1	1.59	1.7
	4	21.3	95.37	0.1	0.1	0.37	0.4
	6	26.6	95.78	0.41	0.4	0.44	0.5

*Unenriched purity for the same sample is averaged across all three sites
**Mean enriched purity is the average purity of enriched samples across three kit lots

REPRODUCIBILITY WITH CLINICAL SPECIMENS

Three lots of the EasySep™ Human Bone Marrow CD138 Positive Selection Kit were tested on multiple myeloma patient bone marrow aspirates (BMA) for performance in downstream FISH assays. A total of 9 clinical multiple myeloma BMA were tested, where each specimen was split in half to test enrichment with two different EasySep™ Human Bone Marrow CD138 Positive Selection Kit lots. The percentage of abnormal nuclei were analyzed using five FISH probes which detect common multiple myeloma chromosomal abnormalities, including the t(11;14) translocation (CCND1/IGH XT), Chromosomes 5, 9 and 15 aneusomies (D5S23, D5S72, CEP 9, CEP 15) and 13q14 deletion.

The percentage of cells with an abnormal FISH signal pattern(s) for each probe are listed in Table 10 and have been colored gray. Three BMA gave normal FISH signal patterns for all five probes tested. One BMA was abnormal for the t(11;14) translocation and four BMA had abnormal signal patterns for the 13q deletion. In all nine BMA tested, the percentage of abnormal cells for each probe were very similar between the plasma cells enriched with the two EasySep™ CD138 kit lots. The same normal/abnormal disposition for a given probe was concordant between the pairs of EasySep™ Human Bone Marrow CD138 Positive Selection Kit lots. Cells which are gray in Table 10 were dispositioned as abnormal with abnormal FISH signal patterns. Cells which are white in Table 10 were dispositioned as normal with FISH signal patterns that did not exceed the cutoffs for percentage of abnormal nuclei. In all cases, normal/abnormal disposition was in agreement indicating that the EasySep™ kit is able to produce reproducible FISH results when used on the intended use specimen.

Table 10. FISH Results Using Five Probes on Multiple Myeloma BMAs Following Enrichment Using the EasySep™ Human Bone Marrow CD138 Positive Selection Kit

Sample No.	Kit Lot	t(11;14), % Abnormal	Chr.5 Trisomy	Chr.9 Trisomy	Chr.15 Trisomy	Chr.5 Tetrasomy	Chr.9 Tetrasomy	Chr.15 Tetrasomy	Chr.5 Monosomy	Chr.9 Monosomy	Chr.15 Monosomy	13q Deletion
1	1	0	25.5	14	4.5	6.5	1	1	0.5	2	3.5	4
1	2	0	27	18	6.5	1.5	2	0.5	1.5	1	1	5
2	1	0	0	88	0	0	1.5	0	0	0	10	96
2	3	0	1	87.5	0	0	0.5	0.5	1.5	0	7.5	95.5
3	2	0	74.5	55.5	49	0.5	0	0	0	0	0	84.5
3	3	0	72	59	46.5	0	2.5	1	0	0	0	80
4	1	0	2	1	1.5	0	0	0	2.5	2.5	1	2
4	2	0	3	3	1	0	0	0	1.5	4	5.5	2.5
5	1	0	9.5	5.5	6.5	0.5	1	1	6	2.5	5	2.5
5	3	0	3	4.5	2	0.5	0.5	0.5	2	2	4	2.5
6	2	0	2	0	53	0	0	0	0.5	0	1	96.5
6	3	0	0.5	1.5	38.5	0	1	0	0.5	2	3	95.5
7	1	89	0	2	2	0	0	0	0	5.5	4	90.5
7	2	90	2.5	7	2	0	0	0.5	2	1	4	91
8	1	0	0.5	1.5	1	0	1	0	2	1.5	4	1
8	3	0	1.5	0.5	1	2.5	0	0	1.5	1	3	0.5
9	2	0	63	53	35	5	1.5	4.5	1.5	1	0	2
9	3	0	60	48.5	38.5	1	0	0	1	1.5	3.5	1

PRECISION

A precision study was performed where, on three non-consecutive days, a bone marrow specimen from a healthy donor was spiked with the CD138+ cell line SK-MM-2 at a low (< 3%) and a medium (3 - 15%) initial CD138+ frequency to generate two separate contrived sample panel members. Two operators performed EasySep™ enrichments on each of the study days, with each operator enriching from one of the two panel members. Each operator performed three runs of enrichments in quadruplicate on one panel member using one kit lot.

Table 11 shows the unenriched and enriched CD138+ cell purity for each of the three runs from all panel members. The enriched purity repeatability precision estimates (%CV across four replicates within a run, n = 4 per run, 3 runs per panel member) ranged from 0.81 - 1.56% and 0.17 - 1.41% for low and medium initial CD138+ frequency panel members, respectively, indicating that enriched CD138+ cell purity variability is well-controlled within run.

Table 11. Unenriched and Enriched CD138+ Cell Purity by Run for Low and Medium Initial CD138 Frequency Panel Members as Determined by Flow Cytometry Analysis

Initial CD138 Frequency Category	Panel Member	Run	Unenriched Purity* (% CD138+)	Enriched Purity (%CD138+)					
				Mean	Min	Max	SD	CV	N
Low	B1	1	1.51	86.39	85.28	87.48	1.01	1.17	4
		2		84.41	83.42	86.35	1.32	1.56	4
		3		83.92	83.05	85.10	1.00	1.20	4
	B4	1	1.32	86.7	85.65	87.13	0.70	0.81	4
		2		86.65	85.87	87.93	0.90	1.04	4
		3		86.96	85.92	87.60	0.76	0.87	4
	B5	1	1.37	82.27	80.99	83.50	1.19	1.44	4
		2		82.74	81.93	83.77	0.77	0.93	4
		3		82.22	81.20	83.54	0.97	1.18	4
Medium	B2	1	10.84	93.65	92.94	95.62	1.32	1.41	4
		2		94.1	93.57	95.05	0.69	0.73	4
		3		93.71	92.75	94.50	0.78	0.83	4
	B3	1	9.84	95.77	95.39	96.50	0.51	0.54	4
		2		95.78	94.81	96.65	0.78	0.81	4
		3		95.54	94.92	96.14	0.69	0.73	4
	B6	1	10.17	92.78	91.10	93.49	1.13	1.21	4
		2		93.62	93.49	93.81	0.16	0.17	4
		3		93.55	92.99	94.21	0.56	0.60	4

* Mean unenriched purity measured across three runs

Table 12 shows the CD138+ cell recovery for low and medium initial CD138+ frequency panel members and the corresponding statistical analyses. Low initial CD138+ frequency panel members had CD138+ cell recoveries ranging from 34.98% - 76.69% (Mean = 53.42%). Medium initial CD138+ frequency panel members had CD138+ cell recoveries ranging from 36.89% - 65.16% (Mean = 50.62%). The overall CD138+ cell recovery within-laboratory precision estimates (%CV across three runs with four replicates, n = 12 total) ranged from 5.52% - 27.4% and 10.53% - 18.32% for low and medium initial CD138+ frequency panel members, respectively.

Table 12. CD138+ Cell Recovery for Low and Medium Initial CD138 Frequency Panel Members

Initial CD138 Frequency Category	Panel Member	Unenriched Purity* (% CD138+)	CD138+ Cell Recovery (%)					
			Mean	Min	Max	SD	CV	N
Low	B1	1.51	55.30	34.98	76.69	15.15	27.4	12
	B4	1.32	48.88	36.63	64.37	7.92	16.2	12
	B5	1.37	56.09	50.56	63.00	3.09	5.52	12
Medium	B2	10.84	50.78	37.58	58.57	6.64	13.07	12
	B3	9.84	49.04	36.89	57.99	5.16	10.53	12
	B6	10.17	52.04	37.88	65.16	9.53	18.32	12

* Mean unenriched purity measured across three runs

LIMIT OF DETECTION

Limit of Detection (LoD) with Contrived Samples

The study aimed to determine the lowest initial CD138+ frequency that gives consistent abnormal FISH results in a contrived specimen after CD138+ cell enrichment using the EasySep™ Human Bone Marrow CD138 Positive Selection Kit. On six non-consecutive days, a whole blood (WB) or bone marrow (BM) sample from a healthy donor was spiked with the CD138+ cell line SK-MM-2 at 20% (acceptable range 26% -14% by flow cytometry) CD138+ initial frequency and then serially diluted by a factor of two with WB or BM to achieve ~20%, 10%, 5%, 2.5%, 1.25%, 0.63%, 0.31%, 0.16%, 0.08%, and 0% (i.e., WB or BM not spiked with SK-MM-2 cells). Each sample was enriched in duplicate using the EasySep™ Human Bone Marrow CD138 Positive Selection Kit and all unenriched and enriched specimens were assessed by flow cytometry and/or had t(11;14) disposition analyzed by FISH.

Table 13 shows the results from the EasySep™ CD138 Limit of Detection Study including the unenriched and enriched CD138+ cell purity measured by flow cytometry and the t(11;14) disposition analyzed by FISH. All contrived specimens spiked with SK-MM-2 cells, targeting as low as 0.08% initial CD138+ frequency, resulted in abnormal t(11;14) disposition in FISH analysis. The enriched CD138+ purity measured by flow cytometry positively correlated with the percentage of abnormal nuclei observed in downstream FISH analysis. The average enriched CD138+ purity that resulted in abnormal t(11;14) disposition in FISH was 33% CD138+, enriching from specimens with an average unenriched purity of 0.08% CD138+. For 0.00% initial target CD138+ frequency specimens, four out of the six donors resulted in normal t(11;14) disposition in FISH analysis, however one BM and one WB donor only had one replicate deemed normal and the other replicate inconclusive due to insufficient number of cells recovered. In conclusion, the EasySep™ CD138 Limit of Detection Study showed the EasySep™ Human Bone Marrow CD138 Positive Selection Kit was able to consistently enrich from contrived specimens with initial CD138+ frequency of 0.05% - 25.06% and achieved consistent abnormal FISH patterns. The limit of detection for the EasySep™ Human Bone Marrow CD138 Positive Selection Kit was set at 0.05% initial CD138+ frequency.

Table 13. EasySep™ CD138 Limit of Detection Flow Cytometry and FISH Analysis Result Summary

Target CD138+ Initial Frequency	Flow Cytometry Analysis								FISH Analysis				
	Unenriched CD138+ Purity (%)				Enriched CD138+ Purity (%)				%Abnormal Nuclei and t(11;14) Disposition				
	Mean	Min.	Max.	N	Mean	Min.	Max.	N	Mean	Min.	Max.	Normal/ Abnormal	N
0.00%	NA	ND	<LoQ	ND: 5 <LoQ: 1	13.33	<LoQ	32.48	12	0.0	0.0	0.0	Normal Inconclusive	N=10 N=2
0.08%	0.09*	0.05*	0.15*	6	33.03	6.31	68.07	12	51.7	30.5	73.0	Abnormal	12
0.16%	0.18*	0.10*	0.30*	6	42.57	11.52	76.2	12	68.0	40.0	82.0	Abnormal	12
0.31%	0.32*	0.21*	0.40*	6	55.42	20.73	83.82	12	83.4	73.5	90.5	Abnormal	12
0.63%	0.69	0.41	0.94	6	67.2	30.97	90.12	12	87.6	80.0	92.5	Abnormal	12
1.25%	1.38	0.87	1.86	6	76.55	51.55	90.43	12	93.1	85.5	97.0	Abnormal	12
2.50%	2.17	1.41	2.84	6	87.07	76.24	93.86	12	95.8	89.5	99.0	Abnormal	12
5%	5.28	3.37	7.25	6	91.71	88.26	95.01	12	97.0	93.5	100.0	Abnormal	12
10%	10.71	8.16	13.21	6	94.39	91.2	96.91	12	98.0	96.5	99.5	Abnormal	12
20%	20.01	14.73	25.06	6	95.01	92.93	98.2	12	98.1	96.5	100.0	Abnormal	12

ND - Not detected; results <LoB.
<LoQ = CD138+ cell detected; Results <LoQ.
NA - Not applicable. Not all specimens had unenriched purity >LoQ

CD138 (SK-MM-2) Flow Cytometry Assay Detection Limits
LoB = 0.04%CD138+
LoD = 0.05%CD138+
LoQ = 0.25%CD138+
*Theoretical unenriched CD138+ purity was reported as the measured purity was <LoQ. Theoretical unenriched CD138+ purity was calculated based on two-fold serial dilution of the lowest unenriched purity >LoQ.
LoB - Limit of Blank; LoD - Limit of Detection; LoQ - Limit of Quantitiation

CONFIRMATION OF LIMIT OF DETECTION (LoD) WITH CLINICAL MULTIPLE MYELOMA BONE MARROW SPECIMENS

The goal of this study was to verify the LoD at 0.05% initial CD138 frequency, determined in the previous LoD study on contrived specimens in one clinical specimen using two kit lots, having three replicates per kit lot. CD138+ cell enrichment was performed on a clinical specimen produced by spiking a multiple myeloma patient bone marrow aspirate into a healthy donor bone marrow aspirate at 0.05% initial CD138 frequency. The clinical specimen was washed and split into six fractions for EasySep™ CD138 cell enrichments, using two kit lots with three replicates per kit lot. Enriched samples were split into two fractions: one fraction was used for plasma cell purity assessment by flow cytometry and the second fraction was used for FISH.

Table 14 shows plasma cell purity before and after enrichment using two kit lots of the EasySep™ CD138 Kit with three replicates per kit lot. The specimen was enriched from 0.05% to a range of 4.1% to 7.4%. Overall enriched purity CV was 19.7%, passing the acceptance criteria of overall CV ≤ 35%. All enriched samples had normal FISH signal patterns for Chromosome 5 and 15 polysomy (D5S23, D15Z4), MYC breakapart, and the CCND1/IGH XT probes; and were abnormal for Chromosome 13q deletion (D13S319) and TP53. The enriched samples' FISH disposition was the same as the undiluted clinical specimen's FISH disposition, with 100% of the replicates being abnormal for Chromosome 13q deletion (D13S319) and TP53.

Table 14. Summary of Limit of Detection (LDT) Results for Clinical Specimens

Sample	Unenriched Plasma Cell Purity (% per 100,000 events)	Enriched Plasma Cell Purity by Flow Cytometry				Genomic Abnormality by FISH					
		% Plasma Cell per 100,000 events	Mean	Stdev	%CV	D5S23	D15Z4	MYC	D13S319,13qter	TP53, D17Z1	IGH@ CCND1 (LDT)
Undiluted Multiple Myeloma BMA	12.9	-	-	-	-	Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 1 - A	0.05	4.1	6.2	1.2	19.7	Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 1 - B		7.4				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 1 - C		7.0				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 2 - A		5.8				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 2 - B		7.0				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 2 - C		5.8				Normal	Normal	Normal	Abnormal	Abnormal	Normal

ANTICOAGULANT INTERFERENCE

Three fresh clinical multiple myeloma bone marrow specimens were recruited for this study. One specimen was processed at ~20 hours post collection, one specimen was processed at ~24 hours post collection, and the third specimen was processed ~22 hours post collection. Each specimen was split into two fractions. One fraction was treated with PBS (the control), while the other was spiked with 3x excess of sodium heparin. Single replicates of each fraction were then washed and enriched using the EasySep™ Human Bone Marrow CD138 Positive Selection Kit. CD138 purity was determined by flow cytometry and then compared to determine whether excess heparin caused interference with CD138 enrichment and purity. A second study evaluated a total of three clinical specimens prepared by adding multiple myeloma bone marrow mononuclear cells into healthy donor bone marrow aspirates at < 0.2% initial CD138 frequency. Three replicates were tested per specimen per condition (i.e. with/without excess sodium heparin). The enriched purities of sodium heparin spiked specimens were not statistically different from that of the control specimens in each study (one tailed t-test, p = 0.1971 and p = 0.2740, respectively). Results indicated that excess sodium heparin was not found to interfere with CD138+ plasma cell enrichment.

Technical Assistance

NOTE: Please re-order using Catalog #100-1133, a kit that comprises the EasySep™ Human Bone Marrow CD138 Positive Selection Kit (REF 100-0748) and the EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate (REF 100-0749).

This PIS can be provided in paper copy upon request.

For technical support, contact techsupport@stemcell.com or call toll-free either +1.604.877.0713 (Canada) or +1.800.667.0322 (North America). For more information, visit www.stemcell.com.

Deletions, additions, or changes are indicated by the change bar in the margin.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer.

Rx For prescription use only	REF Catalog or reference number	LOT Batch code	 Use-by date: YYYY-MM-DD	 Caution, consult accompanying documents	IVD In Vitro Diagnostic Medical Device
 For storage within temperature limits	 Consult Instruction for Use or electronic Instruction for Use	 Do not use if packaging is damaged	UDI Unique device identifier	CE CE Mark	 Manufacturer
EC REP Authorized EC representative in the European Community	CH REP Authorized representative in Switzerland				

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