Enhance Sensitivity of Multiple Myeloma Testing with Purified CD138 Plasma Cells

Multiple myeloma bone marrow samples typically comprise a mix of non-malignant and malignant cells. It can be challenging to detect the small number of malignant myeloma cells when analyzing samples with a large proportion of non-malignant cells. Purification of plasma cells by CD138 positive selection increases the proportion of malignant myeloma cells in the analyte and thereby enhances the detection of genomic aberrations in the sample. This technical bulletin presents a protocol for the isolation of plasma cells to enhance the sensitivity of downstream analysis such as fluorescence in situ hybridization (FISH), microarray-based assays, genomic sequencing, and gene expression profiling for multiple myeloma testing.

Background

Multiple Myeloma

Multiple myeloma is a form of cancer caused by B cell neoplasia that results in dysregulated production and clonal expansion of malignant plasma cells (cells that express CD138 (Syndecan-1) and are involved in the production of antibodies during an immune response). The disease is characterized by excessive numbers of abnormal plasma cells in the bone marrow and overproduction of both intact monoclonal immunoglobulins and free monoclonal kappa and lambda immunoglobulin light chains.

Genomic Aberration Analysis of Multiple Myeloma Samples

Detection and quantification of CD138+ plasma cells in bone marrow is typically the first laboratory screening method for the disease. Multiple myeloma can be distinguished from other B cell neoplasias by the presence of characteristic chromosome aberrations. These chromosome aberrations include chromosome translocation events in the immunoglobulin heavy chain region that result in oncogene activations, chromosomal deletions (particularly of chromosome 13), and hyperploidy. This information can also be used to classify multiple myeloma into subtypes with different characteristics.

Molecular cytogenetics techniques such as FISH are widely used tools for characterizing multiple myeloma. FISH employs nucleic acid probes to detect and localize the presence or absence of specific DNA sequences on chromosomes. The FISH assay is limited by the ability of the existing nucleic acid probes to detect genomic aberrations. Therefore, other techniques with higher resolution (e.g. microarray-based genomic profiling assays, genomic sequencing, and gene expression profiling) are being explored as additional tests to be used in combination with FISH for multiple myeloma testing.

Enhanced Sensitivity with CD138+ Plasma Cell Enrichment

Multiple myeloma bone marrow samples typically comprise a mix of non-malignant and malignant cells. It can be challenging to detect the small number of malignant plasma cells within the large proportion of healthy B cells and non-malignant plasma cells that exhibit a normal karyotype. To identify and analyze the small fraction of abnormal clones it would be necessary to analyze a large number of cells. However, the CD138 antigen is present on all plasma cells (both non-malignant and malignant cells) but not on mature B cells. This makes the CD138 antigen a suitable selection marker for the enrichment of all plasma cells including the malignant multiple myeloma cells. Plasma cell enrichment by CD138 isolation thereby increases the proportion of malignant myeloma cells in the analyte and enhances sensitivity in detecting genomic aberrations compared to analyzing samples with mixed cell populations. Plasma cell enrichment by CD138 selection is therefore a beneficial step prior to downstream analysis to enhance sensitivity and obtain more reliable FISH, microarray-based genomic profiling, and genomic sequencing testing for multiple myeloma.

Easy, Column-Free Plasma Cell Enrichment

One method to obtain CD138+ plasma cells from bone marrow samples or peripheral blood mononuclear cells is by using an immunomagnetic cell isolation technology such as EasySep™ (see Figure 1). With EasySep™, the desired cells are targeted using antibody complexes recognizing CD138 on the surface of the cell and linking the cells to magnetic particles. The sample is then placed in an EasySep™ magnet, the labeled cells are pulled to the side of the tube, and the unlabeled cells can be simply poured or pipetted off into a new tube.

The enrichment of CD138+ cells with EasySep™ has been shown to be an effective method to obtain plasma cells for downstream multiple myeloma testing. For increased time-savings and minimized sample handling, plasma cell enrichment with EasySep™ can be fully automated with the RoboSep™ instruments. A study to evaluate RoboSep™ for CD138+ plasma cell enrichment found that the instrument reliably sorted plasma cells even when they were found in low frequencies (<2%).

The following section provides a complete protocol for plasma cell enrichment from bone marrow, whole blood, and mononuclear cell preparations for subsequent downstream multiple myeloma testing.
Protocol

PART 1: Source-Dependent Sample Preparation

Sample Source: Bone Marrow

Sample Preparation with EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II - Catalog #17887

Dilute the sample 5- to 10-fold in D-PBS (without calcium and magnesium; Catalog #37350) and mix gently by pipetting up and down. Filter the sample through a pre-wetted 70 - 100 μm strainer (Catalog #27260 or Catalog #27217) to remove bone fragments, cell aggregates, and debris. Transfer the bone marrow single cell suspension into a tube and centrifuge at 300 x g for 10 minutes with the brake off. Carefully remove and discard the plasma, without disturbing the buffy coat/red blood cell pellet, and resuspend to the original sample volume with D-PBS. For samples more than 24 hours old, add DNase I Solution (Catalog #07900) at 100 µg/mL to help reduce cell clumping. DNase I Solution can be added directly to pelleted cells, with gentle mixing, before resuspension.

Transfer a maximum of 4.5 mL of the single cell suspension into a round bottom 14 mL polystyrene tube (Catalog #38008). Add 1X EasySep™ Red Blood Cell Lysis Buffer* at a ratio of 1 part lysis buffer to 1 part sample and mix well. The sample is now ready for manual (Part 2A) or automated (Part 2B) cell separation.

Sample Preparation with EasySep™ Human CD138 Positive Selection Kit II - Catalog #17877

Prepare a mononuclear cell (MNC) suspension from whole bone marrow by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). Alternately, remove red blood cells by lysis using Ammonium Chloride Solution (Catalog #07800). After preparation, resuspend cells at 1 x 10^8 cells/mL in PBS containing 2% fetal bovine serum and 1 mM EDTA and transfer a maximum of 8.5 mL to a 14 mL round bottom polystyrene tube (Catalog #38008). The sample is now ready for manual (Part 2A) or automated (Part 2B) cell separation.

Sample Preparation with EasySep™ Human CD138 Positive Selection Kit II - Catalog #17887

Collect whole blood in a blood collection tube containing anticoagulant. Prepare a peripheral blood mononuclear cell (PBMC) suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801).

After preparation, resuspend cells at 1 x 10^8 cells/mL in PBS containing 2% fetal bovine serum and 1 mM EDTA and transfer a maximum of 8.5 mL to a 14 mL round bottom polystyrene tube (Catalog #38008). The sample is now ready for manual (Part 2A) or automated (Part 2B) cell separation.

Sample Source: Frozen Bone Marrow or Peripheral Blood Mononuclear Cells

Sample Preparation with EasySep™ Human CD138 Positive Selection Kit II - Catalog #17877

Incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes. Filter aggregated suspensions through a 37 μm Cell Strainer (Catalog #27305) for optimal results. After preparation, resuspend cells at 1 x 10^8 cells/mL in PBS containing 2% fetal bovine serum and 1 mM EDTA. Transfer a maximum of 8.5 mL to a 14 mL round bottom polystyrene tube (Catalog #38008). The sample is now ready for manual (Part 2A) or automated (Part 2B) cell separation.

*Note: The EasySep™ Red Blood Cell Lysis Buffer (Catalog #20110) is supplied as a 10X concentrate in the kit. 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.

Samples should be processed within 24 hours after collection for optimal cell separation results.
PART 2B: Automated Plasma Cell Enrichment

The cell isolation step to enrich for plasma cells can be fully automated using the RoboSep™-S (Catalog #21000) and RoboSep™-16 (Catalog #23000) instruments. The instruments perform all cell labeling and separation steps. To use RoboSep™ for CD138+ plasma cell enrichment, select the optimized instrument protocol (detailed in the corresponding Product Information Sheet), load the samples and reagents and start the cell separation process by following the on-screen prompts. 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.

PART 3: Assessing Cell Purity After Cell Isolation

For purity assessment of CD138+ cells by flow cytometry use Anti-Human CD138 (Syndecan-1) Antibody, Clone MI15 (Catalog #60003). Plasma cells express either the κ (Kappa) or λ (Lambda) light chain and cell purity can also be assessed by staining for intracellular κ and λ light chains as described by Ahmann and colleagues. Alternatively you can use markers such as Anti-Human CD38 Antibody, Clone HIT2 (Catalog #60014) and Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018) to detect CD38+CD45 variable cells.

PART 4: Downstream Testing

Enriched plasma cells are ready for downstream testing and analysis using techniques such as FISH, microarray based genomic profiling, and genomic sequencing.

PART 2A: Manual Plasma Cell Enrichment

Follow the specific instructions on the corresponding product information sheet for the labeling of cells with the EasySep™ Selection Cocktail and RapidSpheres™ (Figure 1). For the first step, add the EasySep™ Selection Cocktail and RapidSpheres™ to label the desired cells with an antibody complex against CD138 (Syndecan-1) and magnetic particles. Next, place the sample tube inside the EasySep™ magnet, incubate and then pipette or pour off the supernatant. The desired CD138+ cells will remain in the tube and the unwanted cells will have been poured or pipetted off. Remove the tube containing the desired cells from the magnet and resuspend in the appropriate medium. The isolated cells will contain EasySep™ magnetic particles attached to the surface. However these do not do not interfere with downstream applications such as flow cytometry, FISH and nucleic acid isolation.

For more information and to download the Product Information Sheet, visit www.stemcell.com.

Figure 1. Sample Protocol for Manual Selection of CD138+ Cells Using EasySep™ Positive Selection Kit

CD138+ cells are labeled with antibodies and magnetic particles and separated using an EasySep™ magnet. Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction.

*Times will vary depending on the specific reagent, the isolation protocol, and separation platform.

Figure 2. EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II (Catalog #17887)

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: Red blood cells were removed from the start sample by lysis prior to flow cytometry. For samples with CD138+ starting frequency < 10 - 15%, the CD138+ purity of the isolated fraction may be variable.

PART 4: Downstream Testing

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**Product Listing**

**Cell Separation Products**

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<th>Product</th>
<th>Description</th>
<th>For Processing</th>
<th>Catalog #</th>
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<tr>
<td>EasySep™ Human CD138 Positive Selection Kit II</td>
<td>Enrichment of CD138⁺ (syndecan-1) cells from fresh or previously frozen human bone marrow or peripheral blood mononuclear cells (MNCs) by immunomagnetic positive selection.</td>
<td>2 x 10⁶ cells</td>
<td>17877 17877RF</td>
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<tr>
<td>EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II</td>
<td>Enrichment of CD138⁺ (syndecan-1) cells from fresh bone marrow or whole blood by immunomagnetic positive selection.</td>
<td>60 mL blood or bone marrow</td>
<td>17887 17887RF</td>
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<td>RosetteSep™ Human Multiple Myeloma Cell Enrichment Cocktail</td>
<td>RosetteSep™ Human Multiple Myeloma Cell Enrichment Cocktail</td>
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Note: RF kits correspond to EasySep™ kit with RoboSep™ buffer and RoboSep™ filter tips.

**RoboSep™ Instruments**

RoboSep™-S and RoboSep™-16 fully automate all cell labeling and separation steps of the EasySep™ procedure, minimizing sample handling and freeing up technician time. Setup is simple: just load your samples and reagents and return to separated cells. Isolated cells are immediately ready for downstream applications. Learn more at [www.RoboSep.com](http://www.RoboSep.com).

To see RoboSep™ instruments in action, visit [www.RoboSep.com](http://www.RoboSep.com)

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**Staining Antibodies for Purity Assessment by Flow Cytometry**

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<td>Anti-Human CD38 Antibody, Clone HIT2</td>
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<td>Anti-Human CD45 Antibody, Clone HI30</td>
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**References**