Miltenyi Biotec

Multiple myeloma research

Isolation of CD138$^+$ cells

The leading automated method for purification of CD138$^+$ cells from whole bone marrow samples for multiple myeloma research

What is multiple myeloma

Multiple myeloma (MM) is a hematological disorder of plasma cells in the bone marrow. It is the second most common hematological cancer. MM is characterized by a huge clinical heterogeneity despite the homogeneous morphologic appearance of malignant plasma cells (PCs). The onset of interphase fluorescence *in situ* hybridization (FISH) and genomic microarrays largely improved the detection of genomic (chromosomal) aberrations as well as the identification of some cryptic changes, both being increasingly implemented as markers for MM plasma cells.

Challenges in the identification of malignant plasma cells

In order to properly identify and research malignant PCs, CD138$^+$ cells must be detected and at minimum subjected to FISH analysis, currently one of the leading standards for identification.
FISH analysis and other cytogenetic techniques allow the detection of chromosomal abnormalities and provide the means to determine nuclear topology. Purified CD138$^+$ cells are a prerequisite to heighten the sensitivity of FISH analysis and genomic arrays, or for sensitive downstream molecular read-outs such as those obtained from Single Nucleotide Polymorphism (SNP-) arrays. Myeloma researchers state that it is not acceptable to report FISH results in myeloma samples without enriching PCs or using a method that scores only PCs.[1]

An automated solution for enhanced sensitivity

Given the daily demands of a research lab to provide the most accurate methodology for analysis, there is a need for a platform to provide pure cell populations of CD138$^+$ cells. Whole Blood CD138 MicroBeads, in combination with the autoMACS® Pro Separator, allow standardization of cell separation procedures and safe handling of multiple MM samples. Whole Blood CD138 MicroBeads enable fast isolation of CD138$^+$ cells directly from bone marrow samples, thus minimizing hands-on time and maximizing the yield of target cells. No sample preparation is required, such as density gradient centrifugation or red blood cell (RBC) lysis. Purified CD138$^+$ cells are well suited for FISH analysis and functional or molecular analyses such as SNP-arrays.

Cell separation – a brief methodical overview

The automated cell separation is fast, reliable, and can be used by anyone in a routine research lab requiring processing of multiple samples.

1. CD138$^+$ cells from whole bone marrow samples are magnetically labeled with Whole Blood CD138 MicroBeads.
2. Up to six samples are placed in a cooled Chill Rack to maintain samples at 4 °C.
3. The Chill Rack is placed on the MACS® MiniSampler of the autoMACS Pro Separator.
4. Once the pre-set separation program is selected, the sample is automatically loaded onto the autoMACS Column of the autoMACS Pro Separator.
5. CD138$^+$ cells are magnetically retained within the column, while unlabeled cells are collected in the flow-through as the negative fraction. Hence, this cell fraction is now depleted of CD138$^+$ cells.
6. After retraction of the magnet from the column, CD138$^+$ cells are automatically eluted as the positive fraction and can be immediately subjected to downstream applications.

Benefits of MACS® Technology for the isolation of CD138$^+$ cells

- Increased sensitivity for downstream assays
- Reproducibility and consistency achieved in multi-user settings
- Walk-away sample processing of multiple samples when utilizing the autoMACS Pro Separator
- Reliable technology with consistent performance of reagents
- No sample processing required, e.g., density gradient centrifugation (Ficoll™) or RBC lysis – isolate directly from whole blood or bone marrow samples

Table 1 shows in-house comparison data on cell isolation from PCs in a whole blood sample with Whole Blood CD138 MicroBeads and the autoMACS Pro Separator, compared to a different technology using CD138 magnetic beads. Results using the autoMACS Pro Separator demonstrate superior yield and purity.

	autoMACS Pro Separator	Different automated technology
Original volume and Sysmex® cell count	2 mL blood 1.9×10^7 total cells	5 mL blood 4.5×10^7 total cells
Cell count after RBC lysis	Not required	3.6×10^7 cells
Nucleated cells after separation	1×10^6	4×10^5
Purity of CD138$^+$ cells	95%	90% with contaminating RBCs

Table 1: In-house comparison between different automated cell separation platforms for the isolation of CD138$^+$ cells.

Customer data

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In a study of one hundred human MM samples from bone marrow, we compared the efficiency, performance, purity, ease of use, personnel time, and the quality of DNA after the purification of CD138$^+$ cells. Two methods were compared. In the first method, cells were directly purified from bone marrow samples using Whole Blood CD138 MicroBeads and the autoMACS® Pro Separator. In the second method, mononuclear cells from fresh whole bone marrow were enriched by Ficoll centrifugation, followed by positive selection of CD138$^+$ cells using CD138 MicroBeads in combination with the autoMACS Pro Separator.

The purities were similar for both methods, however, the recovery and performance were considerably better for cells isolated directly from bone marrow, than for cells isolated from mononuclear cells enriched by Ficoll centrifugation.

	Percentage of recovered plasma cells
Directly from bone marrow samples	95%
From pre-enriched mononuclear cell samples (Ficoll)	65%

Table 2: Percentage of cells recovered for FISH analysis following positive selection of CD138$^+$ cells directly from bone marrow or from pre-enriched mononuclear cells by Ficoll centrifugation.

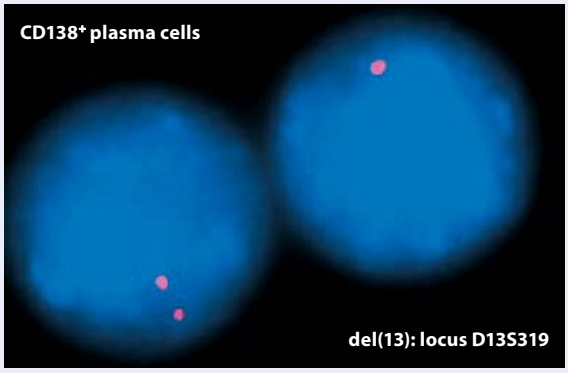
When isolating PCs directly from bone marrow, for 95% of the MM samples we obtained enough cells for the performance of the recommended panel of FISH analyses. For the Ficoll-processed cells we observed inferior performance with very few PCs after isolation, obtaining only 65% PCs.

The quality of DNA was the same for both methods. The isolation of cells directly from bone marrow was 30 minutes faster per sample compared to Ficoll-processed cells. Cells isolated by both methodologies were subjected to FISH analysis.

CD138$^+$ cell enrichment prior to FISH analysis demonstrated the following benefits:

- Greatly increased detection of abnormalities (83% vs. 40%)
- Significant frequencies of abnormalities were observed
- NanoDrop™ analysis of DNA extracted from PCs isolated by Whole Blood CD138 MicroBeads revealed high purity

A



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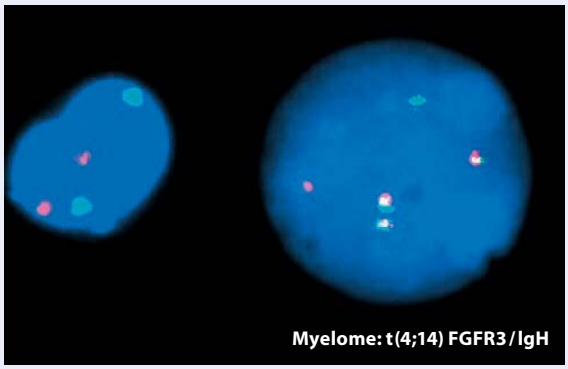

Figure 1: (A) After separation of CD138+ PCs, two interphase cells were hybridized with the LSI D13S319 Single Color Probe. The normal cell (left) shows the two orange signal patterns. The malignant cell (right) shows only one orange signal pattern, due to a deletion event including locus D13S319 or monosomy of chromosome 13.
(B) Two interphase cells, one normal (left) and one malignant (right) were subjected to FISH analysis and hybridized with the LSI IGH/FGFR3 Dual Color, Dual Fusion Translocation Probe (t(4;14)(p13;q32)).
The normal cell shows the two orange (FGFR3) and two green (IgH) signal patterns, respectively. The malignant cell shows an abnormal signal pattern with one orange (FGFR), one green (IGH) and three fusion signal pattern, resulting from a chromosomal translocation event (t(4;14) FGFR3/IgH).

Table 3 shows the detection and frequency assessment of CD138+ PCs prior and post enrichment.
Frequency assessment was determined by flow cytometry using CD138 and CD38 antibodies.

Sample	Detectable PCs in bone marrow samples (%)	CD138+ PCs following isolation (%)
1	2	86.3
2	1	88.9
3	0	91.8
4	5	93
5	5	93.5
6	16	93.7
7	11	95.5
8	24	95.5
9	25	97.3
10	30	97.5

Table 3: Frequency of CD138+ PCs prior to and after enrichment from bone marrow samples using Whole Blood CD138 MicroBeads and the autoMACS Pro Separator. PC frequency was determined by flow cytometry.

Testimonial

Dr. Mossafa, Laboratoire Cerba:
"As a routine multiple myeloma research laboratory, we receive numerous bone marrow samples every day. We must isolate CD138+ cells from these samples to increase the sensitivity of downstream assays such as FISH analysis and whole genome arrays. For this reason, I really required a fast, reliable, standardized method, which allows me to process multiple samples in a convenient way, while maintaining sample integrity.

After testing different technologies, we decided to use Whole Blood CD138 MicroBeads in combination with the autoMACS Pro Separator. From October 2007 to July 2011 we have separated more than five thousand specimens with the autoMACS Pro Separator, as this technology meets all of the requirements of our lab."

Summary

We have developed an SOP for an automated, reliable, and standardized method, which allows the processing of multiple samples in a single day, while maintaining sample integrity and increasing the sensitivity of FISH analysis and whole genome arrays. This workflow solution is being used by renowned routine labs worldwide.

Whole Blood CD138 MicroBeads from Miltenyi Biotec in combination with the autoMACS Pro Separator allow the isolation of PCs directly from bone marrow samples. This technology proved superior to a different automated technology, including less sample manipulation due to the avoidance of RBC lysis, and higher recovery and purity. Moreover there is no need for density gradient centrifugation. The detection rate of chromosomal abnormalities per sample in multiple myeloma and PC dyscrasia significantly improves when analysis is performed on purified populations of high-quality CD138+ PCs.

We thank **H. Mossafa M.D.** and **S. Defasque M.D.** from the Laboratoire Cerba for their contribution of work and the data presented in this report.

Reference

1. http://www.cytogenetics.org.uk/prof_standards/myeloma.htm

Recommended products*	Order no.
Cell separation	
autoMACS Pro Separator – Starter Kit	130-092-197
Whole Blood CD138 MicroBeads	130-093-062
Flow cytometry	
MACSQuant® Analyzer	130-096-343
CD138-PE	130-081-301
CD38-APC	130-092-261
CD19-FITC	130-091-328

*Products are for research use only.

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