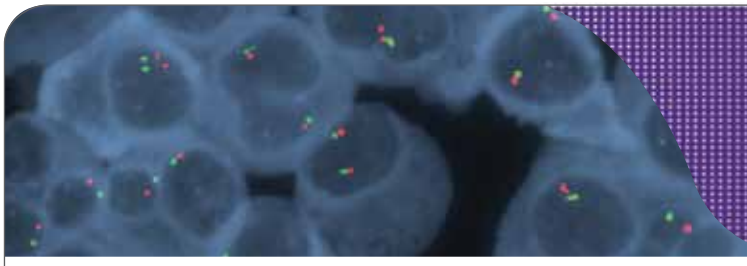

TECHNICAL BULLETIN

Enhance Sensitivity of FISH Analysis with Purified Multiple Myeloma Cells

Purification of multiple myeloma cells by CD138+ positive selection enhances the sensitivity of FISH analysis by increasing the proportion of malignant cells in the analyte.

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)[1]. 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.

Cytogenetic Analysis of Multiple Myeloma

Detection and quantification of CD138+ plasma cells in the bone marrow is typically the first laboratory detection method for the disease. Multiple myeloma can be distinguished from other B cell neoplasias by the presence of characteristic chromosome aberrations; in particular, chromosome translocation events such as oncogene activating translocations in the immunoglobulin heavy chain regions, chromosomal deletions (particularly of chromosome 13) and hyperploidy. This information can also be used to classify multiple myeloma into subtypes with different characteristics[2,3].

There are several challenges in detecting malignant plasma cells in multiple myeloma samples. The slow growth of plasma cells in culture hampers conventional cytogenetic techniques. Additionally, 50 – 70% of multiple myeloma several exhibit normal karyotypes resulting from myeloid cells that are also present in the bone marrow. Conventional cytogenetic techniques can thus fail to detect chromosomal abnormalities[4].

Accordingly, molecular cytogenetics techniques such as fluorescence in situ hybridization (FISH) have become more important tools in the characterization of multiple myeloma. FISH is a cytogenetic technique that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It employs nucleic probe - fluorophore conjugates which hybridize to target chromosomal DNA permitting visualization of the chromosome structure. FISH is a sensitive technique that can detect numerous chromosomal aberrations including submicroscopic deletions. It is currently the most effective technique for identifying genomic aberrations in multiple myeloma. FISH analysis can be performed on cells in interphase (iFISH) circumventing the difficulty of slow plasma cell proliferation that hinders conventional cytogenetics.

Enhanced Sensitivity of FISH Analysis

The majority of mitotic cells in myeloma samples are non-malignant, making it necessary to analyze a large number of cells in order to identify abnormal clones. Samples in which less than 5% of the total cells are plasma cells are difficult to reliably charactarize due to the paucity and fragility of these cells. However, the CD138 antigen is present on all plasma cells (both normal and malignant) but not on mature B cells, making it a suitable selection marker for the isolation of multiple myeloma cells. Enrichment of plasma cells by CD138 positive selection can therefore enhance the sensitivity of downstream FISH analysis[4].

If starting with whole bone marrow or whole blood samples, use EasySep™ Human Whole Blood CD138 Positive Selection Kit (Catalog # 18387). EasySep™ is a powerful immunomagnetic cell separation platform that combines the specificity of monoclonal antibodies with the simplicity of a column-free magnetic system for the isolation of highly purified cells. EasySep™ isolation of multiple myeloma cells can be fully automated with RoboSep™ for increased time-savings and minimized sample handling.

Alternatively, if starting with bone marrow or peripheral blood mononuclear cells (PBMC) samples that have been prepared by Ficoll Paque™ density separation (Catalog #07957), use the EasySep™ Human CD138 Positive Selection Kit (Catalog #18357).

This technical bulletin provides complete protocols for the isolation of multiple myeloma cells from whole bone marrow, whole blood, polymorphonuclear cells (PMNC), or PBMC using EasySep™ or RoboSep™ and subsequent preparation of isolated cells for FISH analysis. For further information, please contact tech.support@stemcell.com.

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Method

PART 1: Sample Preparation

SAMPLE SOURCE	CATALOG #	NOTES ON SAMPLE PREPARATION
Bone Marrow	18387	Remove any clumpy cellular material by filtering the cell suspension through a 70 - 100 µm mesh nylon strainer for optimal results. Transfer the bone marrow single cell suspension into a 14 mL round bottom polystyrene tube (BD, Catalog #352057). Wash the cells with RoboSep™ buffer (Catalog #20104) and resuspend in a volume of buffer equivalent to the volume of the starting bone marrow sample. Add 1X EasySep™ Red Blood Cell Lysis Buffer* at a ratio of 1 part lysis buffer to 1 part sample. Mix well and proceed to Part 2A (manual separation) or Part 2B (automated separation).
	18357	Bone marrow samples can also be lysed of red blood cells using ammonium chloride (Catalog #07800) to obtain a nucleated cell suspension. Alternatively, bone marrow samples can be prepared by Ficoll-Paque™ density separation (Catalog #07957) for a mononuclear cell suspension. Proceed to Part 2A (manual separation) or Part 2B (automated separation).
Peripheral Blood	18387	Collect whole blood in a heparinized blood collection tube. Transfer a maximum of 4.5 mL whole blood to a 14 mL round bottom polystyrene tube (BD Catalog #352057). Add 1X EasySep™ Red Blood Cell Lysis Buffer* at a ratio of 1 part lysis buffer to 1 part blood sample. Mix well and proceed to Part 2A (manual separation) or Part 2B (automated separation).
	18357	Prepare a peripheral blood mononuclear cell (PBMC) suspension from peripheral blood by Ficoll-Paque™ PLUS density separation (Catalog #07957). Proceed to Part 2A (manual separation) or Part 2B (automated separation).
Frozen mononuclear cells	18357	Incubate with 100 µg/mL DNAse (Catalog #07900) for at least 15 minutes at room temperature prior to cell isolation. Proceed to Part 2A (manual separation) or Part 2B (automated separation).

*Note: The EasySep™ Red Blood Cell Lysis Buffer 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.

PART 2A: Manual Multiple Myeloma Cell Isolation

Using EasySep™ bone marrow aspirate or PBMC are labeled with a Tetrameric Antibody Complex (TAC) against CD138$^+$ (Syndecan-1) cells (Figure 1) and dextran-coated magnetic particles. Labeled cells are separated using an EasySep™ magnet (Catalog #18000) without the use of columns. Multiple myeloma cells remain in the tube while unwanted cells are poured off. The sample tube containing the desired multiple myeloma cells is removed from the magnet and the cells are recovered by resuspending them in fresh medium.

The EasySep™ magnetic particles do not interfere with downstream applications. For more information, visit www.stemcell.com to download the Product Information Sheet.

Technical Tip

Samples should be processed within 24 hours after collection for optimal cell separation results. However, CD138$^+$ cells can still be isolated up to 4 days post draw with around 75% purity.

PART 2B: Automated Multiple Myeloma Cell Isolation

Multiple myeloma cell isolation can also be fully automated using RoboSep™ (Catalog #20000). If using RoboSep™, select the optimized instrument protocol, which is detailed on the appropriate Product Information Sheet. All cell labeling and separation steps will be performed by RoboSep™.

To assess purity, stain for intracellular κ (Kappa) and λ (Lambda) light chains (e.g. procedure described within Ahmann GJ et al. Cancer Genet Cytogenet 101(1): 7-11, 1998). Plasma cells express either the kappa or lambda light chain.

FIGURE 1. Selection of CD138$^+$ cells using EasySep™

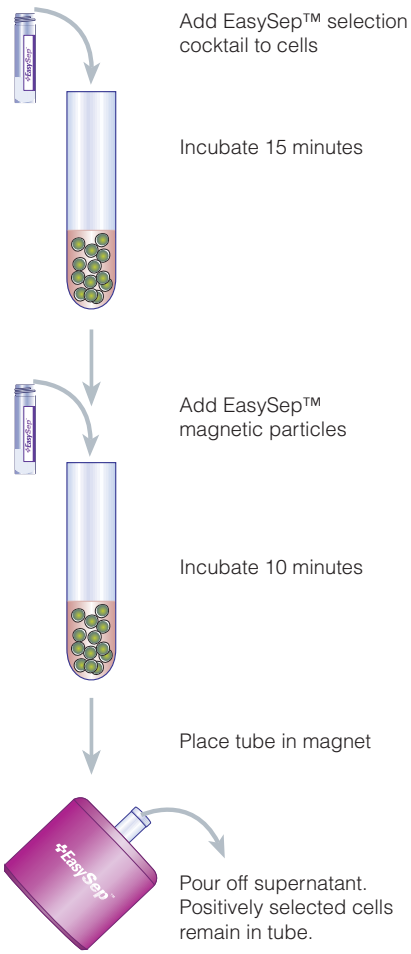
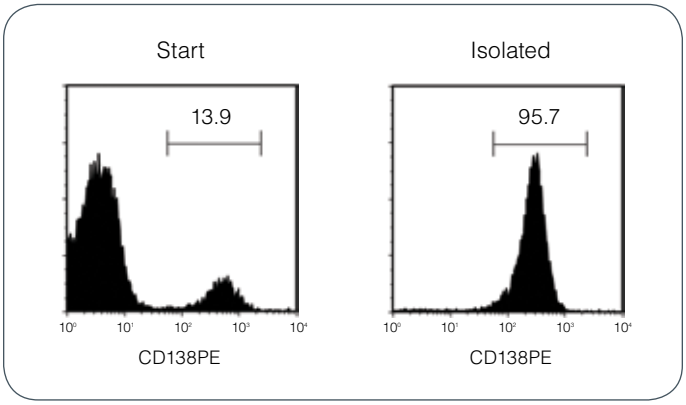


FIGURE 2. FACS Histogram Results for EasySep™ Human Whole Blood CD138 Positive Selection Kit



Whole Blood CD138 positive isolation Data from Mar 13, 2006. U266 tumor cells spiked into whole blood Typical purity range is 89-98% CD138$^+$.

*Red blood cells were removed by lysis prior to flow cytometry.

PART 3: Preparing Purified Multiple Myeloma Cells for FISH Analysis

Isolated multiple myeloma cells are first concentrated onto a cytospin slide using a cyto-centrifuge system. Visual inspection of the slide under a light microscope is necessary to ensure that the cells are of good morphology and that there is minimal cellular overlap. The slide is then blocked in a saline-sodium citrate solution at 37°C and fixed by treatment with a series of increasing percentage ethanol solutions.

In order to allow efficient nucleic probe hybridization, both the cellular chromosomal DNA and the appropriate fluorescent nucleic probes are first denatured. The single stranded probes are then immediately applied to the prepared slide, covered with a cover slip, sealed with rubber cement and incubated in a warm humidified chamber overnight.

After incubation, the cover slip and rubber cement are removed and the slide is briefly heated and washed in saline-sodium citrate buffer solution. The slide is then rinsed with a mild non-ionic detergent solution before drying and staining with DAPI1 (to visualise nucleic chromatin.) The slide is now ready for FISH analysis using a high-quality dual pass fluorescence microscope.

For a more detailed procol, see:

- F. Yao-Shan; Methods in Microbiology. Vol 204. Molecular Cytogenetics. Protocols and Applications.

Product Listing

PRODUCT	CATALOG #	DESCRIPTION	CAPACITY
EasySep™ Human CD138 (Syndecan-1) Positive Selection Kit	18357	EasySep™ Human CD138 Positive Selection Cocktail EasySep™ Magnetic Nanoparticles	for 2 x 10^8 cells
	18347	Starter Kit (includes 3 x 18357 and an EasySep™ Magnet)	for 6 x 10^8 cells
RoboSep™ Human CD138 (Syndecan-1) Positive Selection Kit	18357RF	18357 with RoboSep™ Buffer and RoboSep™ Filter Tips	for 2 x 10^8 cells
EasySep™ Human Whole Blood CD138 Positive Selection Kit	18387	EasySep™ Human Whole Blood CD138 Positive Selection Cocktail EasySep™ Magnetic Nanoparticles EasySep™ RBC Lysis Buffer	for 60 mL blood
RoboSep™ Human Whole Blood CD138 Positive Selection Kit	18387RF	18387 with RoboSep™ Buffer and RoboSep™ Filter Tips	for 60 mL blood
RosetteSep™ Human Multiple Myeloma Cell Enrichment Cocktail	15129	RosetteSep™ Human Multiple Myeloma Cell Enrichment Cocktail	for 40 mL bone marrow
	15169	5 x 15129	for 200 mL bone marrow

RoboSep™: Fully Automated Cell Separation

RoboSep™ is the first instrument to offer true walk-away automation of immunomagnetic cell separation by performing all EasySep™ cell labeling and magnetic separation steps. Set-up is simple: just load your samples and reagents and return to separated cells.

To see RoboSep™ in action, visit www.robosep.com

References

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4. Chen ZJ. Mol. Diag. 7: 560-565, 2005

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