

B Cell Isolation for More Sensitive Array-Based CLL Assays

Introduction

B cell chronic lymphocytic leukemia (CLL) is the most common type of leukemia in the world. CLL affects B cell lymphocytes and causes malignant cells to proliferate and accumulate in the bone marrow and peripheral blood.^{1,2} These malignant CLL cells are distinguished from normal B lymphocytes by the expression of the CD5 surface marker, in addition to CD19, CD20, and CD23.¹ They also carry genetic abnormalities, which can differ among subtypes of CLL and among different leukemic subpopulations in the same individual.^{3,4}

CLL is a highly variable disease and can be divided into a number of subtypes based on the distribution of genetic abnormalities present in the malignant cell population. Since these abnormalities can indicate the course that the disease will take, it is essential that CLL assays are able to identify all underlying mutations with the highest-possible sensitivity and accuracy.^{5,6} In this document, we discuss the advantages of B cell isolation in increasing the sensitivity of array-based CLL assays.

Array-Based Assays for CLL

Array-based comparative genomic hybridization (array-CGH) and array-based single-nucleotide polymorphism analysis (array-SNP) are powerful, efficient, and increasingly common tools for profiling genetic abnormalities in CLL and other hematological malignancies.⁷⁻⁹ Array-based CLL assays have several advantages over older cytogenetic techniques, such as fluorescence *in situ* hybridization (FISH). For example, it is possible to screen tens of thousands of loci in parallel using arrays, whereas FISH is typically limited to a few probes. Similarly, arrays allow mutations to be detected at single-nucleotide resolution, whereas FISH and conventional cytogenetic techniques can detect only gross chromosomal mutations. Recent research shows that array-based assays can detect abnormalities missed by FISH.⁹⁻¹⁴ However, the sensitivity of these arrays is contingent upon starting with a purified cell population and, thus, upstream sample preparation.

B Cell Isolation for Increased Cytogenomic Assay Sensitivity

The purpose of molecular assays for CLL is to detect or quantify a specific DNA sequence or RNA transcript in the malignant B cell population. Therefore, the sensitivity and accuracy of the assay increases with the proportion of B cells in the cell population used

for DNA/RNA extraction. It has been shown that enriching blood samples for B cells improves the sensitivity of molecular assays because it reduces the effects of normal clone contamination caused by DNA from non-B cells.¹⁵ Furthermore, B cell enrichment allows more appropriate comparisons of log₂ ratios among samples by eliminating variability caused by differences in B cell frequency.^{9,16} For example, Hagenkord & Chang (2009) compared array-SNP results on a sample before and after B cell enrichment using RosetteSep™, a reagent for isolating cells directly from whole blood. They showed that enriching B cells before array-SNP allows aberrations that would otherwise have been missed to be detected (Figure 1).⁹

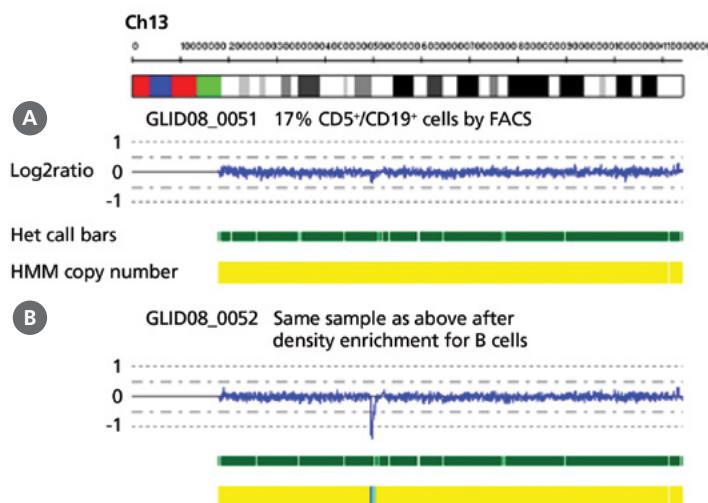


Figure 1. 13q14 Regional Copy Number Heterogeneity and Efficacy of B Cell Enrichment

(A) SNP array-based karyotype of the 13q14 locus of a CLL sample with 17% CD5+/CD19+ CLL cells by flow cytometry. (B) Array-based karyotype of the same sample as (A), but enriched for B cells before DNA extraction, revealing a 13q14 aberration that appears partially heterozygously deleted (light blue) and partially homozygously deleted (dark blue). Dark blue indicates copy number of zero, light blue indicates copy number of one, and yellow indicates copy number of two. The log₂ ratio plot is shown as a smoothed average over 10 SNPs. Figure and caption adapted from Hagenkord & Chang (2009) with permission from Macmillan Publishers Ltd.

In another publication, Hagenkord et al. (2010) used array-SNP to analyze 6 paired samples that were either enriched for B cells using RosetteSep™ or left unenriched. They found that array-SNP detected lesions that would have been missed by FISH and noted the importance of B cell enrichment as a sample preparation step, particularly for samples with a low tumor burden.¹⁶

"Virtually all tumor samples contain some 'normal clone contamination' from non-neoplastic cells. This will dampen the signals from the tumor and cause failure of both copy number and genotyping algorithms... In our hands, enrichment for B cells not only evens the playing field with regard to comparing log2 ratios between samples, but also resolves lesions in samples with low tumor burden or small subclones."

Hagenkord et al. 2010

As a final example, Patel et al. (2008) compared array-CGH using RosetteSep™-enriched CLL samples to FISH using non-enriched samples.¹⁷ In several cases, array-CGH detected aberrations that were missed by FISH (Table 1). Furthermore, Patel et al. noted that their assay was twice as sensitive as previous studies had reported, which they attributed to enriching B cells prior to DNA extraction. These studies highlight the benefits of B cell isolation in improving the sensitivity of molecular assays for CLL detection.

Table 1. Abnormalities Detected by FISH Versus Array-CGH Analysis

Case Number	Abnormalities Detected	
	FISH	Array-CGH
25	34	35
6	0	2
31	34	37

Data from Patel et al. 2008

"...our clonal detection cutoff value of 25% is two times more sensitive than what has been previously reported. This increase in sensitivity is most likely due to using DNA from an enriched population of cells in our study. Therefore, these results suggest that for CLL samples, enriching for B-cells allows for higher sensitivity for detecting clonal abnormalities using array-CGH analysis."

Patel et al. 2008

Summary

When the starting frequency of B cells in a sample is low, the sensitivity of array-based assays for CLL detection can be impacted. The publications mentioned above show that incorporating B cell isolation into the sample preparation step can significantly improve the sensitivity and consistency of molecular assays for CLL, thereby ensuring accurate testing results. To facilitate CLL research, STEMCELL Technologies offers efficient and easy-to-use immunomagnetic and immunodensity selection platforms for B cell isolation that can be easily incorporated into existing protocols.

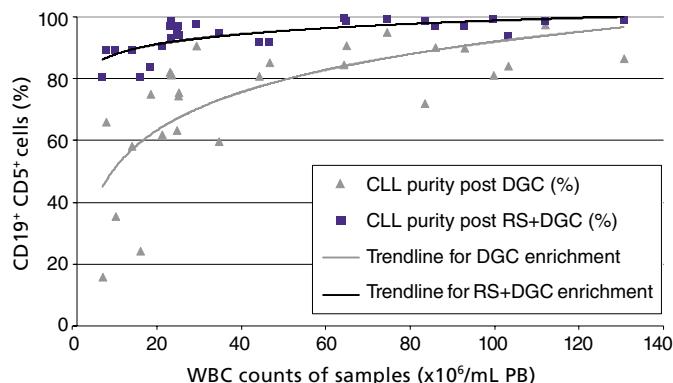


Figure 2. Purity of CD19+ CD5+ CLL Cells Following CLL Isolation

CD19+ CD5+ CLL Cells were isolated by incorporating RosetteSep™ into the density gradient centrifugation step (RS+DGC, purple squares) or were enriched using density gradient centrifugation alone (DGC, grey triangles). Adapted from Essakali et al. 2008, under the terms of the Creative Commons Attribution License.

Fast and Easy Immunomagnetic B Cell Isolation from CLL Samples

EasySep™ is a fast, easy, and column-free system for immunomagnetic cell isolation in as little as 8 minutes. Positive selection protocols can be used to isolate desired cells, while negative selection protocols remove unwanted cells to leave you with untouched B cells for the detection of malignant cells in CLL samples. EasySep™ technology can be used with a variety of downstream applications, including FISH, array-based assays, and next generation sequencing (NGS).

Skip Centrifugation and Isolate Cells Directly from Blood

Obtain untouched, highly purified cells directly from blood without the need for sedimentation or red blood cell lysis using EasySep™ Direct.



Request a Sample
www.stemcell.com/forms/sample-easysep.html

Minimize Sample Handling by Automating Cell Isolation

To increase sample throughput and free up valuable technician time, EasySep™ technology can be automated using RoboSep™ cell isolation instruments. With true walk-away automation, you can isolate B cells from up to 16 samples at once with as little as 5 minutes of hands-on time.



Request a Demo
www.stemcell.com/forms/demo-robosep.html

Table 2. Products for Immunomagnetic Cell Isolation from CLL samples*

Product (Catalog #)	Sample Source
EasySep™ Direct Human B-CLL Cell Isolation Kit (19664)	Whole Blood
EasySep™ Human CD138 Positive Selection Kit II (17877)	Bone Marrow, PBMC
EasySep™ Direct Human CTC Enrichment Kit (19657)	Whole Blood
EasySep™ Human B Cell Enrichment Kit w/o CD43 Depletion (17963)	PBMC
EasySep™ Direct HLA B Cell Isolation Kit (89684)	Whole Blood, Lymph Node, Spleen
EasySep™ Human CD45 Depletion Kit II (17898)	PBMC

Immunodensity B Cell Separation Directly from Whole Blood

RosetteSep™ offers rapid, high-throughput cell isolation directly from human peripheral blood samples in as little as 25 minutes. By crosslinking unwanted cells to red blood cells (RBCs) present in the sample, target cells are purified during standard density gradient centrifugation. To simplify blood layering and ensure sample consistency when isolating cells, RosetteSep™ can be paired with SepMate™, a specialized tube for density gradient centrifugation, and a density gradient medium such as Lymphoprep™.

RosetteSep™ and SepMate™ Procedure (25 min)

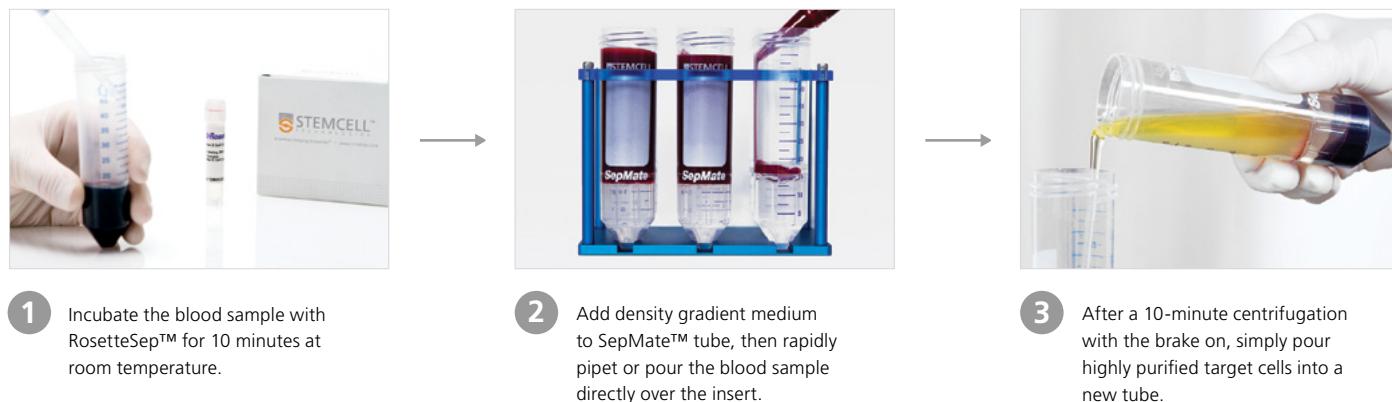


Table 3. Products for Immunodensity-Based Cell Isolation from CLL samples*

Product (Catalog #)	Sample Source
Lymphoprep™ (18060)	-
SepMate™ (85415, 85450)	Whole Blood, Bone Marrow, Cord Blood
RosetteSep™ Human B Cell Enrichment Cocktail (15024)	Whole Blood, Buffy Coat
RosetteSep™ Human Multiple Myeloma Cell Enrichment Cocktail (15129)	Bone Marrow

"The short purification time, the independence from expensive and time-consuming procedures such as FACS and MCS, and the flexible adjustment of cell yields makes [RosetteSep™] an attractive purification method for a wide spectrum of downstream applications... in which a CLL purity of >90% is desirable."

Essakali et al. 2008

*Certain products are only available in select territories. Please contact your local sales representative or the Product & Scientific Support team at techsupport@stemcell.com for further information.

STEMCELL offers several products for B cell isolation from CLL samples, ensuring that an ideal platform is available for every application. To complement our existing products, we also offer custom cell separation kits for your specific needs. To inquire about a custom cell isolation kit, contact techsupport@stemcell.com.

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