

## Zebra Fish Whole Kidney Marrow cells. Myeloid, Precursor, and Lymphoid % after RBC Lysis

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## PRINCIPLE OF THE TECHNIQUE

The Zebrafish is an established model system for studying the embryonic emergence of tissues and organs, including the hematopoietic system. Hematopoietic Stem Cells (HSC) are pluripotent cells that maintain all the differentiated blood lineages throughout the life of an organism. The functional definition of an HSC is a transplanted cell that has the ability to reconstitute all the blood lineages. This designation was established by decades of seminal work in mammalian systems. Zebrafish has emerged as a powerful genetic model to study vertebrate hematopoiesis.

George Streisinger (1981) of The University of Oregon recognized that the Zebrafish, has the following advantages:

- high fecundity (mature females lay several hundred eggs at weekly intervals)
- short generation time (3-4 months)
- external fertilization
- rapid development
- translucent embryos
- easy maintenance

## RESEARCH APPLICATIONS

In contrast to adult hematopoiesis, where committed progenitors are the progeny of HSCs, embryonic hematopoiesis generates committed progenitors before HSCs can be detected. Definitive, or multilineage, hematopoiesis initiates with the formation of committed erythromyeloid progenitors (EMPs) in the posterior blood island (PBI) of the zebrafish embryo. EMPs exist only transiently, and like their counterparts lack lymphoid and self-renewal potential.

Analysis of Zebrafish Whole Kidney Marrow (WKM) or Peripheral Blood (PB) by flow cytometry analysis reveals populations of the major blood lineages, enabling their isolation by Fluorescence Activated Cell Sorting (FACS).

## PROTOCOL

### Standard Procedure:

RBC lysis protocol:

1. Isolate WKM or PB and put into PBS + (0.9X PBS + 5% FCS + 1% P/S)
2. Centrifuge cells at 1500 rpm for 8 min
3. Discard supernatant
4. Resuspend cells in 1mL RBC lysis buffer (see recipe). However, any RBC lysis buffer can be used according to its specifications.
5. Incubate cells at room temperature (RT) for 10 min
6. Stop reaction with 1mL PBS+
7. Centrifuge cells at 1500 rpm for 8 min
8. Resuspend cells in appropriate volume PBS+ for analysis. If analyzing by flow cytometry add a fluorescence marker for dead cell exclusion (ie Propidium Iodide or DAPI).

RBC lysis buffer:

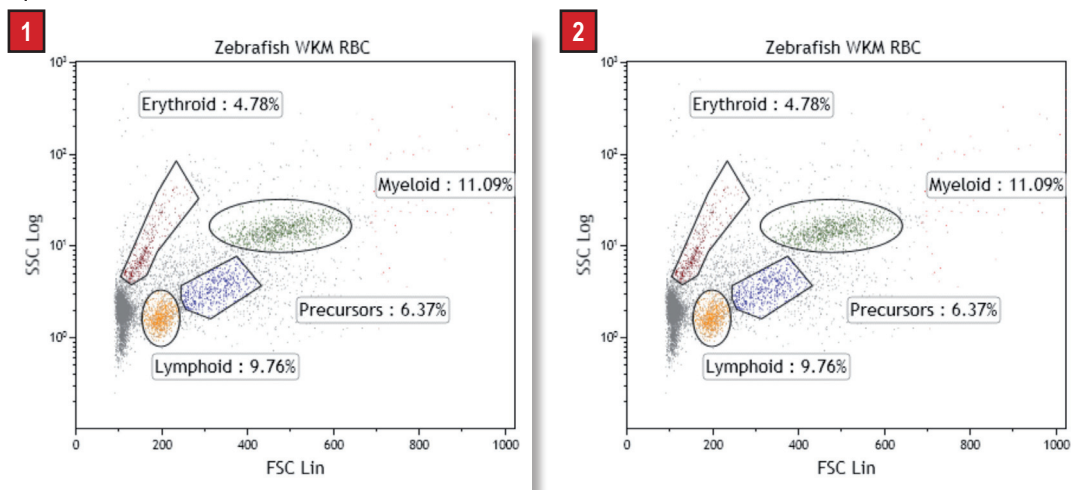
9 mL 0.16M  $\text{NH}_4\text{Cl}$  + 1 mL 0.17M Tris-Cl pH 7.65. Make stock solutions in  $\text{dH}_2\text{O}$ .

Beckman Coulter Gallios\* (3 laser, 10 parameter) settings for WKM analysis of Zebrafish:

- Be aware that it may be beneficial to set the SSC detector to Log for better population separation and less manipulation of Voltage and Gain.
- For acquisition of WKMs, we found that only the SSC parameter needed to be manipulated. However, FSC may need to be adjusted slightly (Voltage=250/ Gain=1.0/Discriminator=100).
- SSC was measured by excitation with the 488 nm laser line and emission detected with a standard 488 nm band pass.
- The SSC voltage detector was set to 637 with a Gain of 50.
- As no FL parameters are being used, compensation is unnecessary.

\* For Research Use Only. Not for Use in diagnostics procedures

## RESULTS



## CONCLUSION

Both the Lymphoid and Myeloid populations are clear and easily distinguishable. However, the precursor population can be variable and difficult to define. For this population, it is best to gate to the right of the Lymphoid population but below the Myeloid population as seen in the diagram above.

Population gating was kept stringent for analysis purposes and threshold was set to exclude background.

*Note: The Erythroid population (Results 2) is non-existent due to RBC lysis procedure (Erythroid depletion) and gate was established from previous sample (Results 1).*

By lysing RBCs, the other major populations (Myeloid and Lymphoid) increase by approximately 50%.

The ability to easily identify and quantitate the lymphoid, myeloid, erythroid, and precursor populations lends to further tracking and cell manipulation. The Zebrafish WKM populations can be irradiated for transplantation studies or transfected with GFP for homing and gene expression research.

## REFERENCES

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3. CD41<sup>+</sup> cmyb<sup>+</sup> precursors colonize the zebrafish pronephros by a novel migration route to initiate adult hematopoiesis. Julien Y Bertrand, Albert D. Kim, Shutian Teng, D.Traver. Development. 2008 May;135(10):1853-62. Epub 2008 April 16.

## NOTES

The results demonstrated in this application sheet represent those generated on the Beckman Coulter Gallios Flow Cytometer. As differences exist in the performance between analyzers, the author cannot guarantee a similar appearance with the use of other flow cytometers. Quality control and assurance were performed to meet manufacturer's specifications using Flow-Check Pro (Beckman Coulter) and 8-peak Rainbow Beads (Spherotech).