




F
l
Micro
w®

Quality Counts!



**Micronucleus
Analysis
Kit**

MicroFlow^{BASIC} (Rodent Whole Blood)



Instruction Manual

For research only. Not for use in diagnostic or therapeutic procedures.

Table Of Contents

1. Materials Provided	2
2. Additional Materials Required	2
3. First-Time Users	2
4. Ordering Information and Technical Services	2
5. Overview of Method	2
6. Introduction	3
7. Collect Whole Blood Samples	4
8. Ship Whole Blood Samples	4
9. Results	4
10. References	5
11. License Agreement and Limited Product Warranty	5

1. Materials Provided

Kit Component	Quantity ^a	Storage Condition
K ₂ EDTA Vacutainer tubes	60	Ambient
Exakt-Pak Shipping Containers	3	Ambient
Foam Cold Packs	6	-10 °C to -30 °C
Thin clear plastic bag for shipping SSF and SPP	3	Ambient
CD-ROM containing: Sample Submission Form (SSF) Study Phase Plan (SPP)	1	Ambient

- a. Each kit provides sufficient materials for the analysis of up to 60 blood samples at Litron.

2. Additional Materials Required

- Refrigerator set at 2 °C to 8 °C
- -10 °C to -30 °C freezer for cold packs
- Shipping forms for overnight delivery service

3. First-Time Users

We strongly recommend reading the entire instruction manual before performing these procedures.

Please do not deviate from the procedures described in this manual. It is important that these steps are followed exactly using the reagents and shipping materials supplied with this kit in order to achieve reliable results. If you have questions, please contact Litron Laboratories by calling (585) 442-0930, faxing us at (585) 442-0934, or sending an email to info@litronlabs.com.

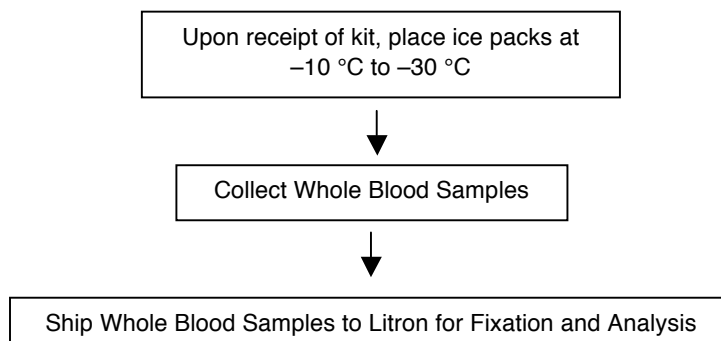
A signed Study Phase Plan is required at Litron before sample analysis is initiated. A Sample Submission Form is also required for each shipment of samples.

4. Ordering Information and Technical Services

Litron Laboratories
 200 Canal View Blvd., Suite 106
 Rochester, New York 14623
 Telephone: 585-442-0930
 Order Toll Free: 877-4-LITRON (877-454-8766)
 Fax: 585-442-0934
 email: info@LitronLabs.com
 World Wide Web: www.LitronLabs.com

5. Overview of Method

The following steps are performed when preparing whole blood samples for shipment to Litron using the MicroFlow^{BASIC} Whole Blood Kit.



6. Introduction

This kit is used when preparing rat or mouse blood samples for flow cytometric enumeration of micronucleated erythrocyte populations. It is ideal for facilities that can collect blood samples and ship them to Litron immediately after collection.

6.1. The Micronucleus Test

The *in vivo* micronucleus test was established as a means of analyzing chromosomal damage. The test is based on the observation that displaced chromatin, resulting from chromosomal loss or breakage, can form a secondary nucleus (micronucleus) outside the daughter nuclei of a dividing cell. Micronuclei (MN) occur spontaneously, but an elevation in the frequency of micronuclei in a population of cells can be indicative of exposure to a genotoxic agent.

Micronuclei are particularly apparent in red blood cells (erythrocytes), which otherwise lack DNA. During erythropoiesis, a hematopoietic stem cell differentiates into an erythroblast and eventually expels its nucleus to become a reticulocyte (RET). The newly formed RET is then released from the bone marrow into the circulating bloodstream, where it develops into a mature normochromatic erythrocyte (NCE). Although the main nucleus is lost during RET formation, MN may be retained in the RET cytoplasm. Peripheral blood is ideal for micronucleus analyses because samples can be obtained from an animal easily and at multiple time points.

6.2. The MicroFlow[®] Method

Litron Laboratories has developed and patented a flow cytometric method to measure micronuclei in both the RET and NCE populations. Unlike mature NCEs, immature RETs are still rich in RNA as well as certain surface proteins (e.g., transferrin receptor, also known as CD71), and can therefore be differentially stained based on these features. An increase in the frequency of micronucleated reticulocytes (MN-RETs) can indicate acute genotoxicity associated with a recent cell division. In mice, an increase in the frequency of micronuclei in the NCE population (MN-NCE) can indicate accumulated DNA damage associated with a sub-chronic or chronic treatment regimen. Elevated MN-NCE frequencies in rat blood need to be interpreted with caution, since splenic filtration function is the dominant factor that influences these values.

The MicroFlow method offers significant advantages compared to traditional microscopic scoring, such as:

- Greater number of cells can be examined for MN
- Faster data acquisition
- Increased statistical power of the assay
- Objective analysis of samples

The MicroFlow method also offers advantages over other automated methods, including:

- Availability for many species of toxicological interest
- Anti-platelet antibody to ensure reliable data
- Biological standards to ensure intra- and inter-laboratory reproducibility of data
- Ability to store samples for extended periods of time before analysis

Crucial components of this method are the biological standards, which aid flow cytometer configuration for the micronucleus scoring application. Fixed blood from animals infected with *Plasmodium berghei* are used to configure the flow cytometer before analysis. Whereas MN are relatively rare and exhibit a heterogeneous DNA content, parasitized cells are prevalent and have a homogenous DNA content. These characteristics make them ideal for calibrating the flow cytometer for the micronucleus scoring application. After optimizing the flow cytometer with the biological standards, micronucleus analyses can be performed reliably and with minimal intra- and inter-experimental variation.

6.3. Regulatory Acceptance

The US FDA accepts preclinical MicroFlow data, and this method adheres to the necessary guidelines as stated by the International Workshop on Genotoxicity Test Procedures (IWGTP). Additionally, the most current Organization for Economic Co-Operation and Development (OECD) guidelines regarding micronucleus testing, Guideline 474, indicates that flow cytometry is an acceptable alternative to manual evaluation.

With regard to rat peripheral blood analysis, Section 4 of the OECD Guideline 474 states "...any appropriate mammalian species may be used provided it is a species in which the spleen does not remove micronucleated erythrocytes or a species which has shown an adequate sensitivity to detect agents that cause structural or numerical chromosome aberrations." Accumulating data suggests that rat peripheral blood is an adequately sensitive compartment for analyzing micronucleus formation, despite the ability of the rat spleen to remove micronucleated erythrocytes from circulation.

7. Collect Whole Blood Samples

If whole blood samples are not fixed at Litron within 24 to 48 hours after collection, the resulting fixed blood samples may be compromised and not compatible with flow cytometric analysis. It is very important to follow the storage (and shipping) instructions provided in this manual.

1. Label each tube with the animal identification number. For FDA GLP analyses, individual samples must be labeled with the following information: Sample ID, Study ID, Date Collected, Source (e.g., mouse or rat) and Type (i.e., Blood). For OECD GLP, label samples with Unique ID and Sample ID.
2. Collect whole blood samples, using common phlebotomy techniques, into the Vacutainer[®] tubes provided.
3. Place the samples in the plastic secondary container and maintain at 2 °C to 8 °C until shipment to Litron Laboratories (same day). If samples are being cooled using ice packs, insulate the tubes appropriately to keep the blood cold, not frozen. Blood must arrive at Litron and be diluted and fixed within 24 to 48 hours after collection.

8. Ship Whole Blood Samples

Ship whole blood samples the same day they are collected for overnight delivery to Litron Laboratories. Trained personnel must follow the applicable guidelines and regulations regarding proper shipping and packaging of whole blood (USDOT, ICAO, IATA 650).

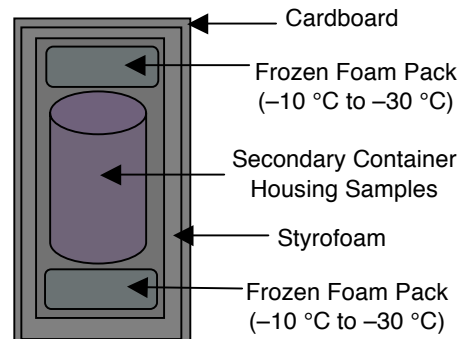
Use the shipping box and cold packs that were provided by Litron. They have been specifically chosen for the purpose of maintaining proper temperatures during transit and to ensure that whole blood samples are received cold (2 °C to 8 °C), but not frozen. Ambient or frozen shipments are not acceptable.

1. Complete Study Paperwork

Complete and sign the appropriate kit-supplied Sample Submission Form and Study Phase Plan and place them inside the thin clear plastic bag. These are necessary for sample analysis.

2. Package the Samples

Ensure that the ice packs are frozen. Place a frozen ice pack in the bottom of the box. Place the secondary container (screw-top wide-mouthed HDPE jar) housing the samples on top of the ice pack. Place the second ice pack on top of the container. See diagram at right.



3. Seal and Label the Box

Place the thin clear plastic bag containing the applicable forms on top of the foam insert. Close the cardboard flaps of the outer box and use shipping tape to secure the middle seam of the box top. Please note, the nomenclature “Diagnostic Specimen” has been replaced by “Biological Substance, Category B”. This wording, along with a UN 3373 label, must be visible on the outside of the box as well as on the air waybill in the “Nature and Quantity of Goods” box.

4. Ship Samples to Litron Laboratories at the following address:

Litron Laboratories
 Attn: Processing Division
 200 Canal View Blvd., Suite 106
 Rochester, New York 14623
 585-442-0930

Unexpected shipping delays may occur at any time. Therefore, it is best to ship samples on Monday or Tuesday and to avoid shipping during holidays.

Immediately after shipping send an email to info@litronlabs.com including your name, telephone number, date of shipment, number of samples, shipping company, and the shipper’s tracking number.

9. Results

Preliminary results will be emailed and a hard copy of the final results will be provided, if requested.

10. References

- Asanami S, Shimono K, Sawamoto O, Kurisu K and Uejima M (1995) *Mutation Research* 347, 73-78.
- Asano N, Torous DK, Tometsko CR, Dertinger SD, Morita T, and Hayashi M (2006) *Mutagenesis* 21(1), 15-20
- Wakata A, Miyamae Y, Sato S, Suzuki T, Morita T, Asano N, Awogi T, Kondo K and Hayashi M (1998) *Environmental Molecular Mutagenesis* 32, 84-100.
- Dertinger SD, Torous DK and Tometsko KR (1996) *Mutation Research* 371, 283–292.
- Dertinger S, Torous D and Tometsko K (January 1999 [filed September 1996]) U.S. Patent No. 5,858,667.
- Dertinger SD, Torous DK and Tometsko KR (August 2000 [filed January 1999]) U.S. Patent No. 6,100,038.
- Dertinger, S. (Patent Pending [filed 6/28/04]).
- Dertinger SD, Torous DK, Hall NE, Tometsko CR and Gasiewicz TA (2000) *Mutation Research* 464, 195–200.
- Dertinger SD, Camphausen K, Macgregor JT, Bishop ME, Torous DK, Avlasevich S, Cairns S, Tometsko CR, Menard C, Muanza T, Chen Y, Miller RK, Cederbrant K, Sandelin K, Pontén I and Bolcsfoldi G (2004) *Environmental Molecular Mutagenesis* 44, 427-435.
- Dertinger SD, Bishop ME, McNamee JP, Hayashi M, Suzuki T, Asano N, Nakajima M, Saito J, Moore M, Torous DK and MacGregor JT (2006) *Toxicological Sciences* 94, 83-91.
- Dertinger SD, Tsai Y, Nowak I, Hyrien O, Sun H, Bemis JC, Torous DK, Keng P, Palis J and Chen Y (2007) *Mutation Research* 634, 119-125.
- Hayashi M, Sofuni T and Ishidate M (1983) *Mutation Research* 121, 241-247.
- Hayashi M, MacGregor JT, Gatehouse DG, Blakey DH, Dertinger SD, Abramsson-Zetterberg L, Krishna G, Morita T, Russo A, Asano N, Suzuki H, Ohyama W and Gibson D (2007) *Mutation Research* 627, 10-30.
- Heddle JA (1973) *Mutation Research* 18, 187–190.
- Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW and Salamone MF (1983) *Mutation Research* 123, 61-118.
- Holden H, Majeska J and Studwell D (1997) *Mutation Research* 391, 87-89.
- Kissling GE, Dertinger SD, Hayashi M and MacGregor JT (2007) *Mutation Research* 634, 235-240.
- MacGregor JT, Wehr CM and Gould DH, (1980) *Environmental Mutagenesis* 2, 509–514.
- MacGregor JT, Bishop ME, McNamee JP, Hayashi M, Asano N, Wakata A, Nakajima M, Saito J, Aidoo A, Moore M and Dertinger SD (2006) *Toxicological Sciences* 94, 94-107.
- Salamone M, Heddle J, Stuart E and Katz M (1980) *Mutation Research* 74, 347-356.
- Salamone MF and Heddle JA (1983) In: F.J. de Serres, ed. *Chemical Mutagens: Principles and Methods for their Detection*, Vol 8. New York: Plenum, 1983; 111-149.
- Schmid W (1975) *Mutation Research* 31, 9-15.
- Serke S and Huhn D (1992) *British Journal of Haematology* 81, 432-439.
- Tometsko AM (July 1993 [filed March 1990]) U.S. Patent No. 5,229,265.
- Tometsko AM, Torous DK and Dertinger SD (1993) *Mutation Research* 292, 129–135.
- Tometsko AM, Dertinger SD and Torous DK (1993) *Mutation Research* 292, 137-143.
- Tometsko AM, Torous DK and Dertinger SD (1993) *Mutation Research* 292, 145-153.
- Tometsko AM, Dertinger SD and Torous DK (1995) *Mutation Research* 334, 9-18.
- Torous DK, Hall NE, Dertinger SD, Diehl MS, Illi-Love AH, Cederbrant K, Sandelin K, Bolcsfoldi B, Ferguson LR, Pearson A, Majeska JB, Tarca JP, Hewish DR, Doughty L, Fenech M, Weaver JL, Broud DD, Gatehouse DG, Hynes GM, Kwanyuen P, McLean J, McNamee JP, Parenteau M, Van Hoof V, Vanparys P, Lenarczyk M, Siennicka J, Litwinska B, Slowikowska MG, Harbach PR, Johnson CW, Zhao S, Aaron CS, Lynch AM, Marshall IC, Rodgers B and Tometsko CR (2001) *Environmental and Molecular Mutagenesis* 38, 59–68.
- Torous DK, Hall NE, Murante FG, Gleason SE, Tometsko CR and Dertinger SD (2003) *Toxicological Sciences* 74, 309-314.
- Torous DK, Hall NE, Illi-Love AH, Diehl MD, Cederbrant K, Sandelin K, Pontén I, Bolcsfoldi G, Ferguson LR, Pearson A, Majeska JB, Tarca JP, Hynes GM, Lynch AM, McNamee JP, Bellier PV, Parenteau M, Blakey D, Bayley J, van der Leede BM, Vanparys P, Harbach PR, Zhao S, Filipunas AL, Johnson CW, Tometsko CR and Dertinger SD (2005) *Environmental Molecular Mutagenesis* 45, 44-55.
- Torous D, Asano N, Tometsko C, Sugunan S, Dertinger S, Morita T and Hayashi M (2006) *Mutagenesis* 21, 11-13
- Torous DK, Dertinger SD, Hall NE and Tometsko CR (2000) *Mutation Research* 465, 91-99.
- Witt KL, Livanos E, Kissling GE, Torous DK, Caspary W, Tice RR, and Recio L (2007) *Mutation Research* 649, 101-113.

11. License Agreement and Limited Product Warranty

By utilizing this kit, your company is agreeing to be bound by the terms of this License. This License allows the use of the MicroFlow[®] Kit for the analysis of 60 samples, either in-house (MicroFlow^{PLUS} Kit), or at Litron's facility (MicroFlow^{BASIC} Kit).

MicroFlow[®]. All rights reserved. *MicroFlow[®]* is a registered trademark of Litron Laboratories. U.S. Patent Nos. 5,229,265, 5,858,667 and 6,100,038. Copyright 2003-2007, Litron.

By accepting these products, you acknowledge that they will be used in accordance with their intended labeling (For in vitro research use only. Not for human or animal diagnostic or therapeutic use.). Uses other than the labeled intended use may be a violation of local laws.

This warranty limits our liability to replacement of this product. Litron shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.