Micronucleus Analysis Kit

MicroFlow^{BASIC} Archive Stage 2
Rodent Fixed Blood

Instruction Manual

For Research Use Only. Not for use in diagnostic procedures.

Table Of Contents

1. Materials Provided

Kit Component	Quantity[a]	Storage Condition
Vacuum-insulated shipping container[b]	1	Ambient
Bag containing icepacks[c]	1	–30 °C to –10 °C
Thin clear plastic bag for shipping Study Phase Plan and Sample Submission Form	1	Ambient

a. Each kit provides sufficient materials for shipping up to 60 blood samples to Litron for analysis.
b. The vacuum-insulated shipping container is designed to ensure that up to 60 samples stored in LTSS will remain sufficiently cold for 48 to 72 hours.
c. Upon receipt, place icepacks in a freezer set to –30 °C to –10 °C.

2. Additional Materials Required

- Previously fixed, frozen rodent blood samples stored at –75 °C to –85 °C in Long Term Storage Solution
- Shipping forms for overnight delivery service
- Freezer set to approximately –20 °C

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 supplies in 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. These forms can be found on Litron's website (www.litronlabs.com).

4. Ordering Information and Technical Services

Litron Laboratories
3500 Winton Place, Suite 1B
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. Introduction

This kit is used for shipping mouse or rat blood samples to Litron for flow cytometric enumeration of micronucleated erythrocyte populations.

5.1. 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 hematopoetic 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.

5.2. 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
- Calibration 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 Calibration 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 Calibration Standards, micronucleus analyses can be performed reliably and with minimal intra- and inter-experimental variation.

5.3. 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, using appropriate calibration standards, can provide better inter- and intra-laboratory reproducibility and sensitivity than manual microscopic scoring. It also states that “Commonly used laboratory strains of healthy young adult animals should be employed. Mice, rats, or another appropriate mammalian species may be used. When peripheral blood is used, it must be established that splenic removal of micronucleated cells from the circulation does not compromise the detection of induced micronuclei in the species selected. This has been clearly demonstrated for mouse and rat peripheral blood.”

6. Ship Blood Samples to Litron

1. Before preparing shipment, verify that all icepacks have been frozen in a freezer set to approximately -20 °C. Please use the shipping container that was provided by Litron. It is imperative that all icepacks are frozen and that the Litron-provided vacuum-insulated shipping container is used in order to maintain correct temperatures during transit.

2. **Complete Study Paperwork and Prepare Shipment**. Complete and sign the appropriate documents and place them inside the thin clear plastic bag. Sample analysis cannot begin until the Sample Submission Form and Study Phase Plan are received.

   Position the following items close to the freezer where samples are stored:
   - Vacuum-insulated shipping container (supplied with kit)
   - Shipping bag (supplied with kit)
   - Frozen Ice packs (supplied with kit)

3. **Place the bagged cryovial storage boxes upright at the bottom of the shipping box** (up to four boxes should fit in one layer on the bottom of the box). Do not ship more than eight cryovial storage boxes in any one box. Fill with frozen ice packs. Place the cardboard piece, silver panel, and foam piece on top of the icepacks (in this order). If you need additional shipping boxes or icepacks, contact Litron.

4. **Seal Box**. Place the thin clear plastic bag containing the applicable forms on top of the foam piece. Close the cardboard flaps of the outer box and use shipping tape to secure the box top. *It is very important to get a tight seal in order to maintain temperatures during transit.*

5. **Ship To Address**. *DO NOT Identify the samples as Category A or B biological substances*. Please verify that the shipper you use guarantees overnight delivery, and ship to the following address:

   Litron Laboratories
   3500 Winton Place, Suite 1B
   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 avoid shipping during holidays.

6. **Send Confirmation**. Immediately after shipping, send an email to info@litronlabs.com including your name, telephone number, date of shipment, and the shipper's tracking number.

7. **Importing to USA**. Track international shipments often to ensure that the samples are not held up at Customs. Several copies of specific Customs forms are usually required. Information regarding importing materials into the United States should be available from your courier, or the United States National Center for Import and Export. As the nature of the samples is defined as a test kit, an import permit is not required, however a USDA inspection may be required. Please contact Litron or the USDA for further information (www.aphis.usda.gov).

7. Results

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

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9. 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).

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.