Micronucleus Analysis Kit

MicroFlow^{BASIC} (Rodent Whole Blood) **Instruction Manual**

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

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1. Materials Provided

Kit Component	Quantity[a]	Storage Condition
K_2EDTA Blood Collection Tubes	15 or 60	Ambient
Anticoagulant/Diluent	10 mL (both kits)	2 °C to 10 °C
Exakt-Pak Shipping Containers	1 or 3	Ambient
Foam Cold Packs	2 or 6	–10 °C to –30 °C
Thin clear plastic bag for shipping required forms (Study Phase Plan and Sample Submission Form – see below)	1 or 3	Ambient

a. Each kit provides sufficient materials for the analysis of up to 15 (Trial kit) or 60 blood samples at Litron.

2. Additional Materials Required

- Refrigerator set at 2 °C to 8 °C
- Heparin-coated capillary tubes (we recommend Fisher Scientific, cat # 22-260-950)
- –10 °C to –30 °C freezer for cold packs
- Shipping forms for overnight delivery service
- The Sample Submission Form and Study Phase Plan are available online (www.LitronLabs.com). The Sample Submission Form and an original, signed Study Phase Plan must be sent with the samples in the shipping box. Analyses cannot be completed prior to receipt of an approved Study Phase Plan.

3. First-Time Users

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

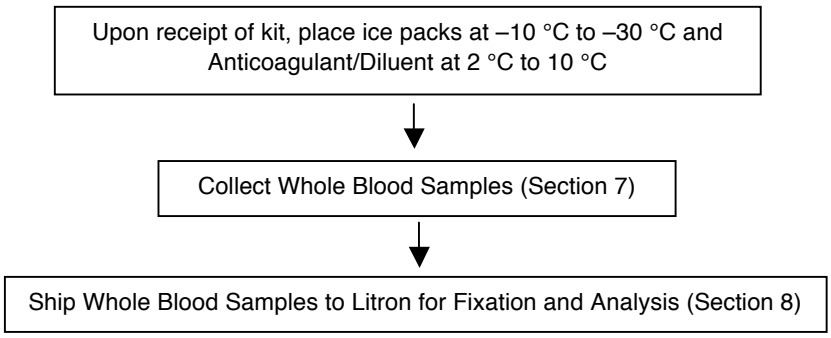
Please do not deviate from the procedures described in this manual. It is important that these steps are followed exactly using the reagents and shipping materials supplied with this kit in order to achieve reliable results. If you have questions, please contact Litron Laboratories by calling (585) 442-0930,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

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. 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 for 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 hematopoetic stem cell differentiates into an erythroblast and eventually expels its nucleus to become an immature erythrocyte [also called 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, 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.

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, 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.”

7. Collect Blood Samples

If 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.

Important Notes:

- Use an IACUC approved method to collect 250 µL to 500 µL blood.
- Whatever blood collection technique is used, it is essential that blood is free-flowing from an animal whose heart is still beating in order to prevent platelet activation and cellular aggregation.
- If blood will be collected by nicking a tail vein with a surgical blade, it is important to warm the animals under a heat lamp for several minutes. Once the blood starts flowing, use heparin-coated capillary tube(s) to collect blood. We recommend heparin-coated capillary tubes from Fisher Scientific, cat # 22-260-950.
- If blood will be collected by submandibular puncture or retro-orbital bleed, use heparin-coated capillary tube(s).
- If blood is collected with a small gauge needle and 1 cc syringe, first coat the inside of the needle/syringe with several hundred µL of kit-supplied Anticoagulant Solution. Then, hold the needle/syringe upright and expel any air that may be in the barrel of the syringe. Proceed by discharging the Anticoagulant Solution from the syringe. For most needle and syringe combinations, this will leave behind approximately 50 to 60 µL Anticoagulant Solution in the so-called dead volume.

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 free-flowing blood sample (see Important Notes, above). IMMEDIATELY transfer the blood sample to appropriately labeled EDTA tube as described below. Repeat for additional samples.

   a. In cases when blood is collected into heparin-coated capillary tubes, transfer the entire volume of blood into the bottom of an EDTA tube and gently pipette up and down 3 times to mix.

   b. In cases when blood is collected into Anticoagulant Solution-coated needle/syringe, transfer the entire volume of blood into the bottom of an EDTA tube. It is important to open the caps on the EDTA tubes as opposed to puncturing the septum with the needle. Once blood is added to a tube, make sure the tube is tightly recapped for transport.

3. Blood can be maintained in EDTA tubes at ambient temperature for up to 2 hrs. For longer periods of time, maintain EDTA tubes at 2 °C to 10°C (not on ice as this may result in cellular lysis).

4. Transfer EDTA tubes containing blood samples to Exakt-Pak jar and proceed with packaging and overnight shipment (FedEx “Priority Overnight” or comparable).

5. Transfer EDTA tubes containing blood samples into the plastic secondary container (Exakt-Pak jar) and maintain at 2 °C to 8 °C until shipment to Litron Laboratories (same day for overnight delivery – FedEx “Priority Overnight” or comparable). Blood must arrive at Litron and be diluted and fixed within 24 to 48 hours after collection.

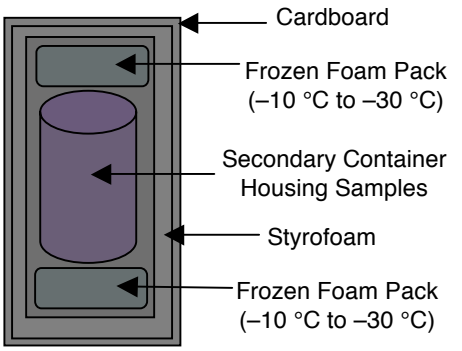
8. Ship Blood Samples

Ship 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 ºC to 8 ºC), but not frozen. Ambient or frozen shipments are unacceptable.

1. Complete Study Paperwork

Complete and sign the appropriate 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
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 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.

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- Section 4 of the OECD Guidelines for the Testing of Chemicals: Mammalian Erythrocyte Micronucleus Test, Guideline 474 (Adopted 26th September 2014).
- Where applicable, GLP regulations for non-clinical laboratory studies as developed by the FDA (21 CFR 58). Please note that the computerized systems utilized for data acquisition, data analysis and report generation have undergone an internal validation guided by FDA GLP regulations. Litron is working towards 21 CFR part 11 compliance.
- Where applicable, Principles of GLP by OECD [C(97)186/FINAL].
- Where applicable, ISO 10993-3: Biological evaluation of medical devices – Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicology (2003-10-15).
- Where applicable, ICH Harmonised Tripartite Guideline: Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, S2(R1), current *Step 4* version dated 9 November 2011.

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 15 (Trial Kit) or 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.