Litron Laboratories

In Vitro Micronucleus

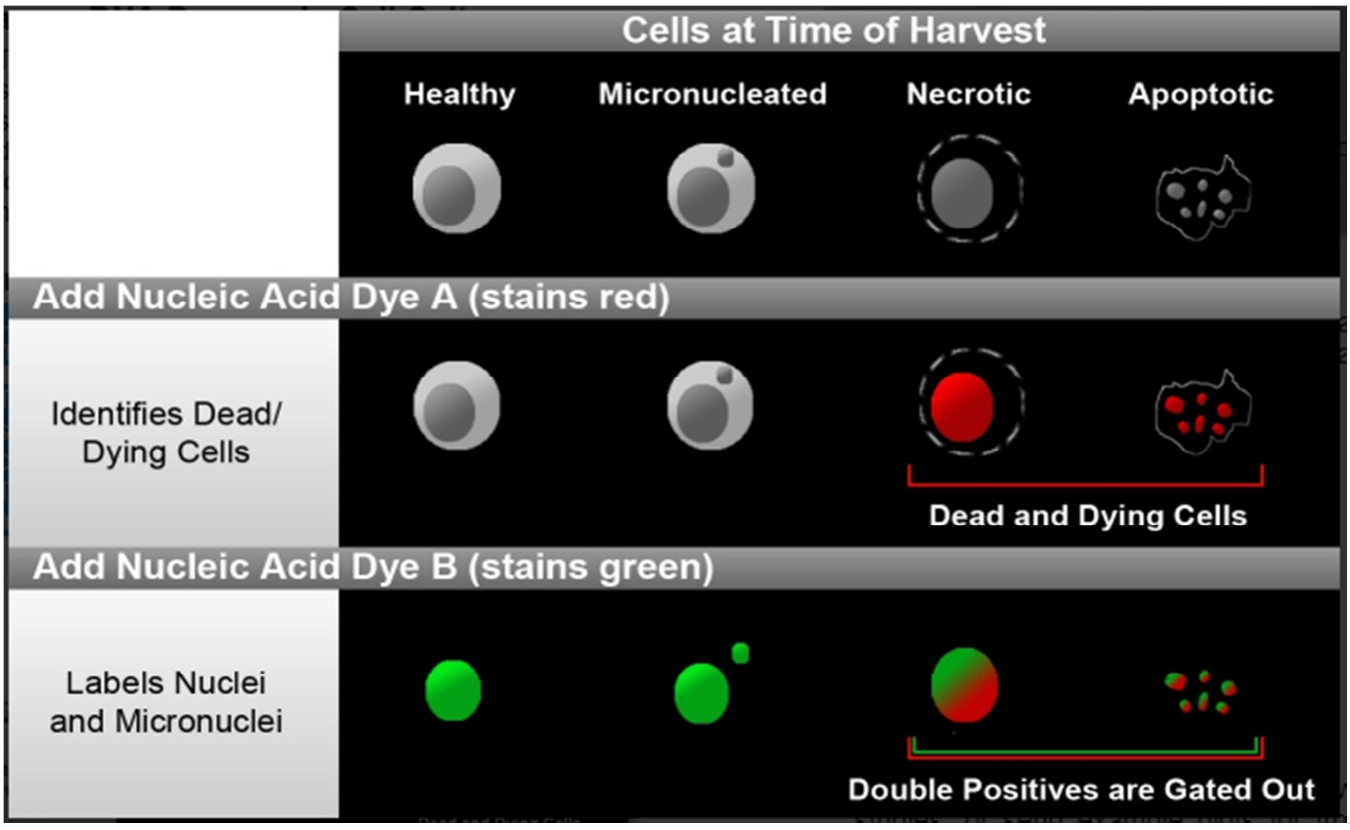
Measure DNA Damage in Cell Cultures

This test detects damage to the chromosomes, or spindle apparatus, of cells. After exposure to a test substance, cells are allowed to divide. It is upon division that this type of damage can result in the formation of a smaller ‘micronucleus', apart from the main nucleus.

MicroFlow® Kits

Litron's MicroFlow kits use flow cytometry to analyze micronuclei *in vitro*.

The key element of this method is the sequential staining. One staining step identifies dead and dying cells. The second step lyses cells and stains DNA. This approach allows you to distinguish micronuclei from other events, such as apoptotic bodies



Automation

Hundreds of samples analyzed in a few hours. Eliminates tech time with "walk away" analysis using auto samplers.

High Content

Obtain these additional endpoints:

- Relative survival
- Membrane integrity
- Cell cycle
- Mode of Action
- Low dose threshold (NOEL)

Unlimited Technical Support

Get answers to your questions, help with validation studies, or send example plots for troubleshooting. We're always here to assist you.

Cell Lines

Use a variety of attachment and suspension cell lines, including:

- L5178Y
- CHO-K1
- TK6
- V79
- WIL-2
- HepG2

Skin models and other types of cells and tissues are in development.

Specifications

In Vitro MicroFlow Kits

Available in Three Sizes

- **250/50**: Analyze 250 samples in 96-well plates or analyze 50 samples in larger format plates or flasks.
- **1,000/200**: Analyze 1,000 samples in 96-well plates or analyze 200 samples in larger format plates or flasks.
- **2,000/400**: Analyze 2,000 samples in 96-well plates or analyze 400 samples in larger format plates or flasks.

Shipping Conditions of the Kit

- Ambient

Storage Conditions of Kit Components

- Freezer (-20 °C)
- Refrigerator (4 °C)
- Ambient

What's Included in the Kit

- **Incomplete Lysis Solution 1**
  Lyses cells after staining
- **Incomplete Lysis Solution 2**
  Lyses cells after staining
- **Nucleic Acid Dye A**
  Stains the DNA of cells undergoing apoptosis or necrosis
- **Nucleic Acid Dye B**
  Stains nuclei and micronuclei
- **RNase Solution**
  Removes RNA so Nucleic Acid Dye B stains only nuclei and micronuclei
- **10x Buffer**
  Used in the lysis solutions

Additional Materials Required

- Cell line and cell culture materials
- Freezer (-20 °C) and refrigerator (4 °C)
- Centrifuge and other general lab supplies
- Flow cytometer capable of 488 nm excitation
- Light source (fluorescent preferred)
- If using 96-well plates, an 8-channel bridge and aspirator manifold (available from V&P Scientific)

Regulatory Information

In Vitro MicroFlow Kits

Can I use the in vitro micronucleus test as part of the standard battery?

Yes. ICH states, “Several *in vitro* mammalian cell systems are widely used and can be considered sufficiently validated: The *in vitro* metaphase chromosome aberration assay, the *in vitro* micronucleus assay and the mouse lymphoma L5178Y cell *Tk* (thymidine kinase) gene mutation assay (MLA). These three assays are currently considered equally appropriate and therefore interchangeable for measurement of chromosomal damage...”

MicroFlow In Vitro *Kits use flow cytometry to analyze hundreds of samples in just a few hours. This method supports both attachment and suspension cell lines. Contact us for more information on how* In Vitro *MicroFlow kits can support your regulatory submissions*.

In Depth

In Vivo MicroFlow Kits

This assay simultaneously provides the following genotoxicity and cytotoxicity measurements:

Genotoxicity Measurements

- **% Micronucleus Formation**
  Micronucleus frequency provides information on induction of chromosome damage.
- **Mode of Action**
  When used with certain cell lines (e.g. hamster CHO-K1), the percentage of hypodiploid nuclei can provide information regarding mode of action, discriminating aneugens from clastogens.

Cytotoxicity Measurements

- **Cell Number**
  By including latex ‘counting beads’ in the cell culture, relative survival, relative increased cell counts and relative population doublings can be determined. These measurements are useful for setting top concentration of test article.
- **Dead/Dying Cells**
  The health of treated cells can be determined by the percentage of particles that are stained with Nucleic Acid Dye A (EMA-positive). Any dose where the EMA-positive fold increase (over background) is higher than the MN fold increase should be considered too cytotoxic to interpret.
- **Cell Cycle Information**
  Changes to the cell cycle can be determined from DNA-associated fluorescence. The detection of the G2/M delay, associated with a DNA damage response, can help assure you have reached a proper dose.