Miltenyi Biotec

In Vitro MicroFlow® Kits and MACSQuant® Analyzer 10

High-throughput genotoxicity assays

In cooperation with

Litron Laboratories

Introduction

For preclinical safety assessments in compound screening, mutagenic test systems, such as the *in vitro* micronucleus assay, are an established part of the safety testing procedures used to analyze the potential risk for genetic damage. They provide information on chromosomal damage in cultured or primary cells by detecting the formation of small membrane bound DNA fragments (micronuclei) in the cytoplasm of interphase cells. Traditionally, the *in vitro* micronucleus assay requires a microscopic analysis of the treated samples which limits its applications to high-throughput compound screening.

The *In Vitro* MicroFlow® Kit (Litron Laboratories) in combination with the MACSQuant® Analyzer 10 provide a fast, standardized, and automated flow cytometry–based workflow that overcomes the limitations of microscopic analysis by providing high-content information which is both reproducible and reliable.

The *In Vitro* MicroFlow Kits are standardized kits that enable flow cytometric detection of micronuclei derived from both adherent and suspension cultured cells. It allows for the sequential staining and liberation of nuclei and micronuclei from each sample, prior to the analysis on a flow cytometer. When conducted following proper procedures, results from the *In Vitro* MicroFlow Kits are suitable for regulatory safety submissions.

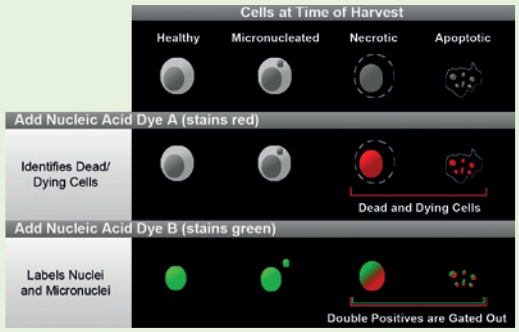

Figure 1: *In Vitro* MicroFlow kit sequential staining procedure.

The MACSQuant Analyzer 10 flow cytometer is a platform that can deliver high-throughput, high content information on genotoxicity when combined with the *In Vitro* MicroFlow Kits. The MACSQuant Analyzer 10 provides automated sampling for 96-well plate analysis and is equipped with a syringe needle capable of volumetric pipetting, thereby enabling absolute counting. In addition, the easy-to-use MACSQuantify™ Software (21 CFR part 11 compliant) provides direct access to instrument parameters for data collection and immediate analysis of results. Paired with the *In Vitro* MicroFlow Kit assay, pre-made templates and settings are available for download, significantly reducing, or even removing, set-up, and optimization time.

In Vitro MicroFlow Kits	MACSQuant Analyzer 10
Specialized – Assay developed from the ground up for flow cytometric detection of micronuclei	**Powerful** – 96 well plate processing
Specific – Sequential staining process allows distinction between micronuclei and other apoptotic bodies	**Reproducible** – Sample autolabeling reduces manual errors and hands-on time
Versatile – Compatible with adherent and suspension cell lines	**Traceable** – 21 CFR part 11 FDA compliant software
Trusted – Suitable for regulatory safety submission	**Precise** – Absolute cell counts give you even more reliable results compared to manual counting methods
Established Workflow used worldwide	

Results

To demonstrate the compatibility of the MACSQuant® Analyzer 10 with *In Vitro* MicroFlow Kits, human lymphoblastoid TK6 cells were exposed to a range of concentrations of methyl methanesulfonate (MMS), a well-known chromosomal damaging agent. After incubation, the samples were processed with the *In Vitro* MicroFlow Kit and subsequently analzyed by flow cytometry using the MACSQuant Analyzer 10 with the MACSQuantify™ Software.

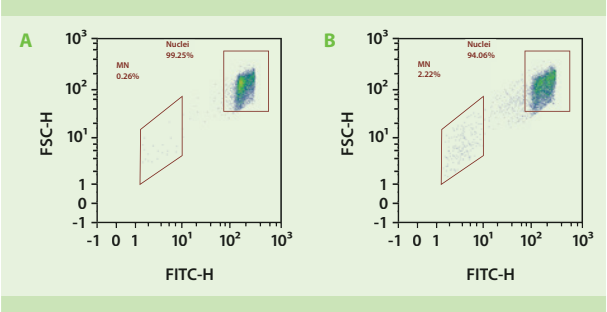

Figure 2: Micronuclei (MN) are readily discriminated from whole nuclei via the staining procedure using the *In Vitro* MicroFlow Kit and visualized and enumerated by the MACSQuant Analyzer 10. The dot plot data are derived from TK6 cells exposed to a vehicle control sample or to MMS. The increased number of events in the MN gate is clearly evident indicating the genotoxic activity of the compound.

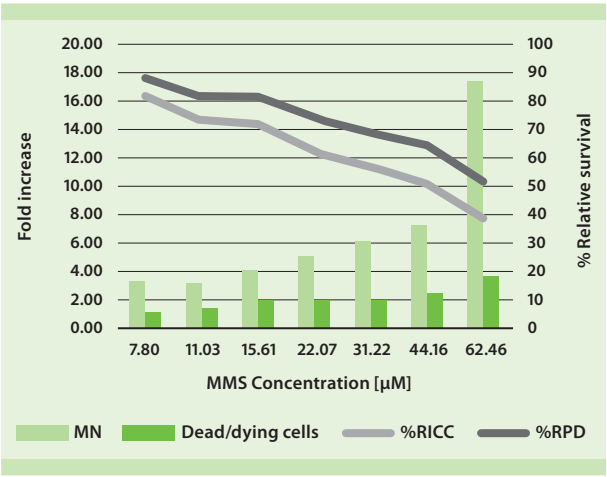

Figure 3: The micronucleus response was tested across the full dose range of MMS, revealing a dose-dependent increase in combination with increased cytotoxicity. The results were analyzed (including increase in MN), in dead/dying cells, and the relative survival as calculated from relative increased cell counts (RICC) and relative population doubling (RPD).

Conclusions

The results demonstrate that the combination of the *In Vitro* MicroFlow Kits with the MACSQuant Analyzer 10 enable a rapid, simple collection of chromosomal damage information that is required for preclicinal safety studies. This methodology provides a much faster, more objective approach than traditional methods marked by manual scoring. This alignment provides a turn-key solution for automation and activation for easy conversion from current methods and marks the advent of an improved methodology for genetic toxicology screening.

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