Micronucleus Test - A Convenient Solution Using Assay Ready V79 Cells

summary

The micronucleus test is a method frequently used in toxicology to detect genotoxic effects. During cell division, genotoxic chemicals result into the formation of micronuclei, which are used as a measure for the genotoxic damage. The OECD recommends several rodent cell lines for this assay, such as V79. Here, we demonstrate that the micronucleus test can be reliably performed with cryopreserved V79 cells, which are used instantly from a frozen vial without prior cultivation. These prequalified Assay Ready Cells display an unaltered sensitivity to clastogen and aneugen chemicals compared to cells, which have been maintained in culture before use. To set up the assay, 3-well chamber slides from Ibidi® were used, which made the assay very convenient. Within the slides, cells can be cultured, treated, fixed and mounted without transfer.

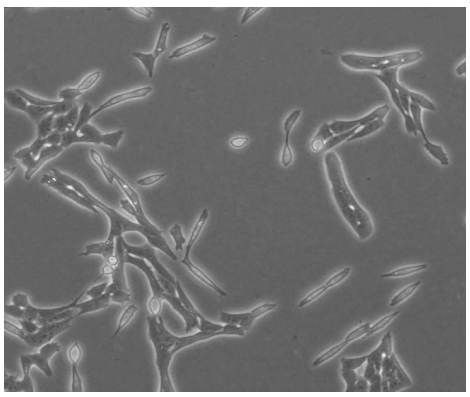

Fig. 1: Assay Ready V79 Cells after 24h incubation.

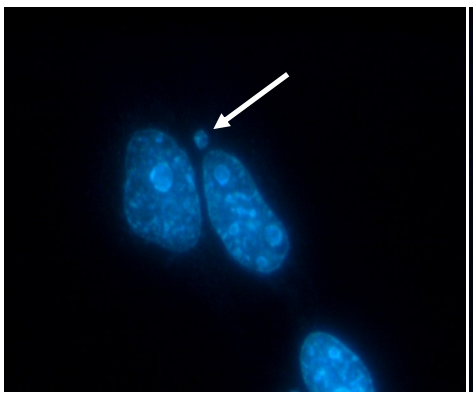

Fig. 2: Micronucleus (arrow) after treatment with 0.2 µg/ml Mitomycin C.

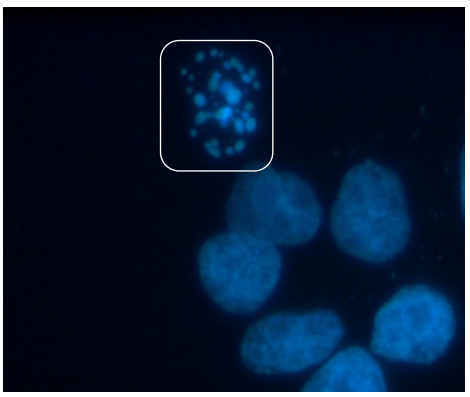

Fig. 3: Apoptotic cell with a completely fragmented nucleus (white box).

introduction

The determination of genotoxicity represents an important element for the safety assessment of chemicals. A battery of *in vitro* tests is available for this, including the micronucleus test. DNA damage can result in double-strand breaks, the formation of chromosome fragments or even result in the loss of entire chromosomes. During mitosis, these fragments are enclosed separately and form micronuclei next to the daughter nucleus (Fig. 2).

Commonly, these tests are conducted with cells that have been maintained in culture for several passages to ensure optimal cell fitness. In recent years, it has been demonstrated for various applications that cryopreserved cells can be used instantly after thawing when optimized freezing protocols are applied. These Assay Ready Cells immediately regain their full function and fitness and do not undergo an extended recovery or lag-phase after resuscitation.

preparation of Assay Ready Cells

V79 is a Chinese hamster lung fibroblast cell line commonly used in genotoxicity testing (Fig. 1). The cells were cultivated in DMEM supplemented with 10% FBS and 2mM L-Glutamine at 37°C in a humidified atmosphere with 5% CO_2. For expansion in T-flasks and CellSTACKs, the cells were gently detached with Accutase at a confluence of 80% and reseeded at 30.000 cells/cm^2.

To prepare an Assay Ready V79 cell bank, the harvested cells were resuspended in cryopreservation medium containing 5% DMSO and automatically dispensed at 5E5 cells/vial using a XSD-Biofill decapping and filling device. Finally, the cells were cryopreserved in a CryoMed 7452 controlled rate freezer at a cooling rate of 1°C per minute and stored in the vapor phase of liquid nitrogen.

micronucleus assay

The OECD guideline recommends assessing laboratory proficiency by using a set of chemicals, which cause chromosome breaks (clastogen, with or without prior metabolic activation) or interfere

EUROPEAN OFFICE

+49 (160) 987 577 56

acCELLerate GmbH
Osterfeldstraße 12-14
22529 Hamburg - Germany

please@accellerate.me

www.accellerate.me

US OFFICE

+1 (732) 698 3404

acCELLerate GmbH
1 Jill Court, Bldg. 16/10
Hillsborough, NJ 08844 - USA

with spindle formation during mitosis (aneugen). Mitomycin C (clastogen), Colchicine (aneugen), and Cyclophosphamide (clastogen after activation) were used in this study. The assay was performed in 3-well chamber slides from Ibidi®, which consist of a self-adhesive and easy to detach silicon gasket mounted on a microscope glass slide for cell cultivation, treatment, and fixation (Fig 4).

One vial of Assay Ready V79 was thawed, and the cells were seeded into 3-well chamber slides at a density of 10.000 cells per well. The cells were incubated at 37°C for 24h to allow them to attach to the glass slide. The medium was removed, and the cells were treated with test chemical dilutions for 16h. After incubation, the cells were fixed with 500µl of a mixture of acetic acid and methanol (1:4), washed once with 500µl cold methanol and finally with 500µl ddH_2O. Fluoroshield™, which contains DAPI to stain the DNA, was used to mount the cells under a glass coverslip. 2.000 cells per well were analyzed by fluorescence microscopy (Ex340nm/ Em488nm). Apoptotic cells, which display a completely fragmented nucleus (Fig 3.), and cells with a micronucleus (Fig. 2) were counted separately to determine the viability and the micronucleus rate. At least three experiments per chemical were conducted in triplicates.

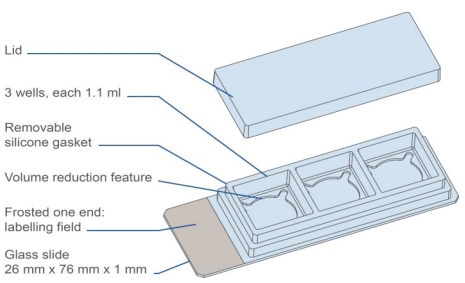

Fig. 4: 3-Well Chamber, Ibidi®

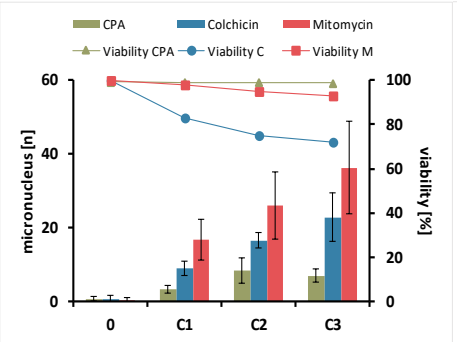

Fig. 5: Dose-dependent micronucleus rate and viability of Mitomycin C, Colchicine, Cyclophosphamide (CPA) with Assay Ready V79.

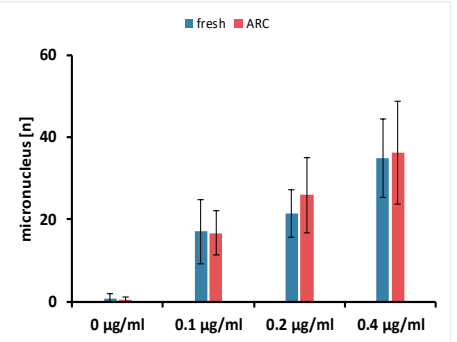

Fig. 6: Dose-dependent micronucleus rate of Assay Ready V79 (ARC) and cells from a continuous culture (fresh) after treatment with Mitomycin C.

A dose-dependent increase in the micronucleus rate was found for all three chemicals (Fig 5). Mitomycin C had the strongest potential to generate micronuclei without having a cytotoxic effect. Colchicine also displayed a significant micronucleus rate, but also an increased level of apoptosis. Cyclophosphamide, which was metabolically activated with S9 (liver extract), showed the least potential to form micronuclei. To compare Assay Ready Cells, with V79 from a continuous culture, cells were treated with Mitomycin C under identical conditions. Both cell types showed nearly the same sensitivity to increasing concentrations of Mitomycin C and were therefore comparable (Fig. 6). Assay Ready V79 as well as continuous cultured V79 displayed very low basal micronuclei rates with 0.6 micronuclei and 1.1 micronuclei respectively on average (n=15).

discussion

The results demonstrate that Assay Ready V79, which are used instantly after thawing, are suitable for use in micronucleus testing. They display a dose-response effect with a very low basal micronucleus rate to all three chemical categories and perform like cells from an ongoing culture. The comparable low basal rates indicate the high standard of cell quality and that there is no previous damage due to the freezing process.

Therefore, by using pre-qualified Assay Ready V79 Cells, which are produced in large homogeneous batches and can be stored in liquid nitrogen for years, the reproducibility of the micronucleus test between individual tests and from lab to lab can be significantly increased. In combination with the 3-well chamber slides from Ibidi®, the well-established micronucleus assay becomes much more convenient because the slides offer an all-in-one solution to cultivate, treat, and mount the cells directly on a standard glass microscope slide.

related products

instaCELL® micronucleus assay kit validated kit with Assay Ready V79 cells, assay buffer, Mitomycin C, Fluoroshield™ and 3-well chamber slides (CatN° SF120-01)

V-79 Assay Ready Cells (Cat. N° RE781)