XENOMETRIX

Swiss Commitment for Bioassays

Rapid MicroNucleus MoA

One–hour FISH MicroNucleus Assay
for a reliable Genotoxicity Assessment

DRAFT **Short Protocol**

Art. No. M020

Art. No. M100

Please note: Items are shipped at ambient temperature with cooling elements. Kit contents will be fully active **if shipment is received within 5 days from dispatch and stored immediately as indicated on the individual items and as described on page 3 of this manual**. If components are damaged please contact Xenometrix by phone: ++41-61-482-14-34; fax: ++41-61-482-20-72, or Email: info@xenometrix.ch

For Research use only **Version 4.051_May 2020**

Rapid MicroNucleus MoA

One–hour FISH MicroNucleus Assay for a reliable Genotoxicity Assessment

M020 – 20 Slides Kit
M100 – 100 Slides Kit
Patent pending

Introduction

Rapid MicroNucleus MoA is designed for an easier and reliable detection of all repeated human centromere sequences with a high efficiency and strong signal. Rapid MicroNucleus MoA permits to identify the nature of substances (or exposure) (Aneugens and Clastogens) in addition to the scoring of micronucleus in line with TG OECD 487 [1].

The cytokinesis-block micronucleus (CBMN) assay is the international method for determining the genotoxic potential of a substance and agent, recommended by the OECD for genotoxicity testing [1]. Micronuclei (MN) can be formed in dividing cells that contain either whole chromosomes or acentric chromosome fragments. The use of CBMN assay combined with fluorescence *in situ* hybridization (FISH) using pan-centromeric probe was proposed to discriminate not only clastogenic effect (MN without centromere staining) to aneugenic effects (MN with centromere staining), but also to increase the sensitivity of this technique [2].

Rapid MicroNucleus MoA provides in one step:

- easy and reliable detection of micronuclei
- the determination of the nature of micronuclei with or without centromere sequences (chromosome lagging and acentric deletions, respectively).

Rapid MicroNucleus MoA can simultaneously (i) detect micronuclei and (ii) improve with a higher precision the nature of exposure.

Rapid MicroNucleus MoA reduces the analysis time while considerably improving its precision compared to conventional and molecular techniques (1 h *Vs.* 16 h).

Rapid MicroNucleus MoA does not suffer of interferences (*e.g.*, detection of RNA, micronuclei with different densities, precipitation, or cytotoxic effects resulting in cytoplasmic alterations).

The kit can also be used to detect induced chromosomal aberrations after exposure to genotoxic agents according to OECD guideline 473 [3].

Changelog:

Date	New version	Changes
12.05.2020	4.05	• First version, based on v4.05 of the IfU.
13.05.2020	4.051	• Added Art. No for the 100-slide kit in the first page • Removed unnecessary material.

Assay Description

CBMN (cytokinesis-block micronucleus) procedure were performed according to standard procedures [1]. Slides were spread and stored at –20°C until use according to the conventional protocols [1]. The Rapid MicroNucleus MoA can also be used in cyclic mononuclear cells prepared according standard protocols (*i.e.*, cell lines).

Of note, the Rapid MicroNucleus MoA kit can be used on any human cells but not on animal cells.

This kit provides all key reagents needed to perform fluorescence *in situ* hybridization for the detection of centromere sequences by microscopy. Rapid MicroNucleus MoA kit is compliant with OECD 487 [1], the slides must be prepared accordingly, with very stringent conditions, to allow accurate detection of centromere sequences.

The probe is added to the slide, denatured and hybridized. The slides are then counterstained with DAPI (4',6-diamidino-2-phenylindole) and the analysis is performed using fluorescence microscopy (FITC or Cy3 staining can be performed).

Kit Components and Storage Conditions

Art. Nr.	Product	Quantity	Storage	Comment
M020-MF20	Microfilm	20 pcs	RT	Ready-to-use.
M020-WA20	Wash Solution 10×	20 mL	2–8 °C	Dilute in distilled ddH_2O.
M020-PR20	Centromere probe	0.4 mL	–20°C	–
M020-DC20	DAPI Counterstaining 1000×	0.1 mL	–20°C	Dilute in distilled ddH_2O.
M020-MS20	Mounting Solution	2× 1 mL	–20°C	Ready-to-use.

Required Equipment and Consumables NOT Included in the Kit

- Phosphate-Buffered Saline (PBS) for washing of slides prior to *in situ* hybridization
- ddH_2O
- Slides
- Ethanol
- Formamide 70% (it can be used as a Washing Solution in case of high background).

- Heating block adjustable to 80°C
- Glass Hellendahl jars (100 mL)
- Glass coverslips (24×60 mm)
- Fluorescence microscopy equipped with filters for FITC or Cy3 and DAPI.

References

1. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg487-2014-508.pdf
2. Vral A., Decorte V., Depuydt J., Wambersie A., Thierens H. 2016. A semiautomated FISH based micronucleuscentromere assay for biomonitoring of hospital workers exposed to low doses of ionizing radiation. *Molecular medicine reports* **14**:103–10
3. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg473-2014-508.pdf

Assay Procedure

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