Rapid MicroNucleus MoA Kit

2 hours FISH Micronucleus Assay for Micronucleus Test or for Chromosomal Aberration

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Short Protocol of

Art. No. M020 – 20 Slides
Art. No. M100 – 100 Slides

Art. No. M020
Art. No. M100

Note 1
Please read the entire manual carefully before starting the experiments!

Items are shipped at ambient temperature with cooling elements. Kit contents will be fully active if shipment is received within 10 days from dispatch and stored immediately as indicated on the individual items and as described on page 3 of this manual. Please note that Xenometrix does not take any responsibility for the product once the product leaves the warehouse. If components are damaged or if any problems occur, please contact Xenometrix AG by phone: +41 61 482 14 34 or Email: info@xenometrix.ch. Please note that complaints of damaged goods are accepted only within 3 days after arrival.

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Changelog

Date	New version	Changes
13.09.2021	5.0	Complete overhaul
17.04.2023	6.0	Specification of handling steps
20.05.2024	7.0	- More detailed description and numbering of handling steps - More specifications on handling steps with Hellendahl Jars, covering Jars during incubation - An additional washing step with PBS introduced - Dehydration and washing steps slightly prolonged resulting in a longer handling of 2 hours - Optional fixing (formamide) and washing steps added for samples with high background - Optional rinsing treatment with formaldehyde - More detailed description of evaluation of the results - Sources indicated for material not provided with the kit
25.06.2024	8.0	- Small corrections

1. Kit Components and Storage Conditions of Products Upon Arrival

Article No.	Description	Quantity	Storage	Description
M020-MF20	Microfilm	22 pieces	RT	Ready-to-use.
M020-WA20	Wash Solution 10× available also supplementary	48 mL	2–8°C	Dilute 1:10 in ddH_2O before use
M020-PR20	Centromere probe	0.44 mL	–20°C	
M020-DC20	DAPI Counterstaining 1000×	0.11 mL	–20°C	Dilute 1:1000 in ddH_2O before use
M020-MS20	Mounting Solution	2× 1.1 mL	–20°C	Ready-to-use.

Preparation of the Reagents

- Wash solution 10×: Dilute 1:10 in ddH_2O (*e.g.*, add 10 mL of Wash Solution 10× to 90 mL of ddH_2O for a final volume of mL).
- DAPI Counterstaining 1000×: Dilute in 100 mL of ddH_2O and protect from light after dilution. Stability after dilution: 6 months at –20°C or 1 month at 2–8°C.
- Mounting Solution (for the fixation of the glass coverslip). Always protect from light and store at –20°C. Acclimatize at room temperature for ca. 15 minutes before use, then move back directly to its recommended storage (do not store on ice!).

2. Required Equipment and Consumables NOT Included in the Kit

- Phosphate-Buffer Saline (PBS) for washing of slides prior to in situ hybridization
- ddH2O
- Slides, e.g. FisherbrandTM Superfrost TM Plus Microscope Slides, Cat. No. 22-037-246
- Slide rack, e.g. Dutscher, Cat. HS15981A
- Ethanol
- Formaldehyde 37%
- Formamide 70% (it can be used as a Washing Solution in case of high background) – see under 2nd Pre-Treatment 5.3.
- Heating block adjustable to 80°C, e.g. Stuart SD160, or Leica Thermobrite for slides
- Glass Hellendahl jars or non-transparent porcelain jars, 100 mL, e.g. Dutscher, Cat. No. BPL018
- Glass coverslips, 24×24 mm
- Fluorescence microscopy equipped with filters for FITC or Cy3 and DAPI, and with objectives with at least 40x magnification

3. Assay Description

Please note that the intent of this kit is to perform the staining procedure after cells are exposed to a test compound.

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

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 for the detection of micronuclei,[1] the slides must be prepared accordingly, with very stringent conditions, to allow accurate detection of centromere sequences.

The FITC labeled centromere probe is added to the slide, denatured, and hybridized to centromere sequences. After hybridization the slides are counterstained with DAPI (4',6-diamidino-2-phenylindole) for the visualization of the DNA. The hybridized probes will emit a fluorescence signal and analysis is performed using fluorescence microscopy.

The kit can also be applied as a part of a workflow that is suitable for the identification of substances that cause structural chromosomal aberrations in accordance with OECD TG 473 [3].

Furthermore, there are several other application areas, where the Kit can be successfully deployed:

- Biological dosimetry [2]
- Investigation of chromosomal rearrangements and genomic instability [5]
- Disease monitoring and identification of patients with increased risk for cancer development [6]
- Occupational, environmental, and dietary studies [7]

Cytochalasin B treatment is recommended in order to have cells completing mitosis during or after the exposure to the test chemical. This facilitates the scoring of micronuclei only in binucleated cells (i.e. those that have completed mitosis). The appropriate concentration of Cytochalasin B is between 3 and 6 µg/mL in line with OECD TG 487 [1]. To test for the efficiency of the cytokinesis block a control can be performed without Cytochalasin B treatment.

Exogenous metabolizing systems should be applied when the cells have inadequate endogenous metabolic capacity with respect to the test chemical in question. The most used system is a co-factor supplemented rodent liver S9 fraction, typically from rat liver. The recommended S9 concentration is between 1 and 2% (v/v), but can be increased up to 10% in the final test medium in compliance with OECD TG 473 / 487 [1][3]

Please note that the S9 fraction and the S9 cofactor solutions kit are not included in the Kit but are available separately from Xenometrix (Phenobarbital/β-Naphthoflavone induced lyophilized rat liver S9, 1 mL, art.no. PRS-PB01or 2 mL, art.no. PRS-PB02 and S9 Cofactor Kit, 25 mL, art.no. PCO-0800).

Please note that the positive controls are not included in the Kit. Recommended positive controls in compliance with OECD TG 473 / 487 [1][3]:

- Clastogenic compounds without metabolic activation
- Methyl methane sulfonate (CAS: 66-27-3)
- Mitomycin C (CAS: 50-07-7)
- 4-Nitroquinoline-N-Oxide (CAS: 56-57-5)
- Clastogenic compounds requiring metabolic activation
- Benzo(a)pyrene (CAS: 50-32-8)
- Cyclophosphamide (CAS: 50-18-0)
- Aneugenic compounds
- Colchicine (CAS: 64-86-8)
- Vinblastine (CAS: 143-67-9)

The Rapid MicroNucleus MoA kit can be used on any human cells but not on animal cells.

As an example, the following cell lines / cell cultures can be used in accordance with OECD TG 473 or 487 :

- Human primary cell cultures (including peripheral blood lymphocytes)
- TK6 lymphoblast cell line

4. 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) (Figures 1–3), but also to increase the sensitivity of this technique.

Rapid MicroNucleus MoA provides in one step:

- direct detection of clastogenic or aneugenic compounds.
- easy and reliable detection of micronuclei
- reduction of the analysis time (2-3 h vs 16 h).
- improvement of precision compared to conventional stain techniques
- no interferences due to RNA, micronuclei with different densities, precipitation, or cytoplasmic alterations.

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

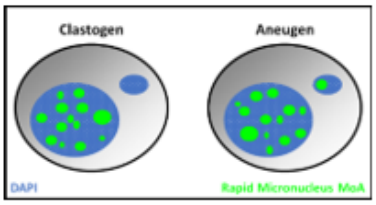

Figure 1. Schematic representation of the readout of the Rapid MicroNucleus MoA kit. DAPI staining alone cannot discriminate whether the Mode of Action of the genotoxic agent is aneugenic or clastogenic whereas centromere staining using Rapid MicroNucleus MoA can easily detect MN with centromere sequences (aneugenic effect) and discriminate MN without any staining (clastogenic effect).

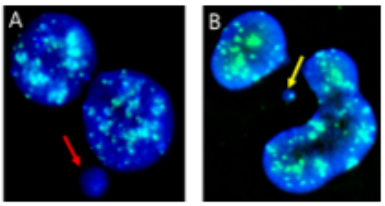

Figure 2. Binucleated cells obtained via the CBNM assay and stained with the Rapid MicroNucleus MoA kit. Centromere sequences (green signal) were stained and nuclei were counterstained using DAPI.
A) Red arrow points micronuclei lacking centromere sequences: Clastogenic effect.
B) Yellow arrow points a micronucleus with centromere sequences: Aneugenic effect.

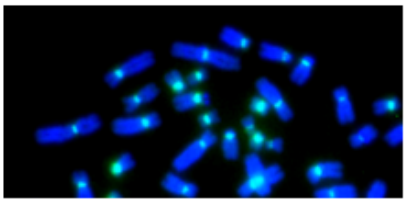

Figure 3. Metaphase staining with the Rapid MicroNucleus MoA kit. All centromere sequences were stained (green signal) and chromosomes were counterstained with DAPI (blue).

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
4. www.metasystems-international.com

Assay Scheme