Rapid MicroNucleus MoA Centromere Telomere Kit

FISH Micronucleus Assay with Centromere and Telomere Probes for Identification of clastogenic/aneugenic Compounds or for Chromosomal Aberration

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Short Protocol

This protocol is a shortened version of the instruction for use for the following kits:

Art. No. MTE020 – 20 Slides
Art. No. MTE100 – 100 Slides

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 2 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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Note 2
After registration on www.xenometrix.ch, all certificates of analysis, Instructions for Use can be downloaded from the protected “Download” area. If you are not registered to the protected area of the Xenometrix homepage, please contact info@xenometrix.ch.

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.
MCT020-WAI20	Wash Solution I	100 mL	2–8°C	Mix 93 mL of Wash Solution I with 217 mL 100% formamide before use.
MCT020-WAII20	Wash Solution II 10x	44 mL	2-8°C	Dilute 1:10 in ddH_2O before use.
MCT020-CPR20	Centromere Probe CT	0.018 mL	–20°C	Dilute 1:100 in PNA Buffer before use.
MCT020-TPR20	Telomere Probe CT	0.018 mL	–20°C	Dilute 1:100 in PNA Buffer before use.
MCT020-PBU20	PNA Buffer	0.33 mL	-20°C	Add 0.77 mL 100% formamide.
M020-DC20	DAPI Counterstaining 1000×	0.11 mL	–20°C	Dilute 1:1000 in ddH_2O before use.
MCT020-MS20	Mounting Solution CT [1]	0.44 mL	–20°C	Ready-to-use.

[1] 1 drop (20 µL) Mounting Solution CT is added to each slide, total volume 0.44 mL

Preparation of the Reagents

- Wash Solution I: Mix 93 mL of Wash Solution I with 217 mL 100% formamide before use.
- Wash solution II 10X: Mix 40 mL Wash Solution II 10X with 360 mL H2O.
- PNA Buffer: Add 0.77 mL 100% formamide. The formamide-complemented PNA Buffer can be stored at -20°C.
- Centromere Probe CT: Dilute 1:100 in formamide-complemented PNA Buffer before use.
- Telomere Probe CT: Dilute 1:100 in formamide-complemented PNA Buffer before use.
- DAPI Counterstaining 1000X: Dilute 1:1000 in ddH_2O, i.e. 100 µL DAPI Counterstaining 1000X in 100 mL H2O and protect from light after dilution. Stability after dilution: 6 months at –20°C or 1 month at 2–8°C.
- Mounting Solution CT (for fixing the glass coverslip and for preserving the fluorescent signal). Always protect from light and store at –20°C. Acclimatize at room temperature for at least 15 minutes before use, then place it back directly to its recommended storage (do not store on ice!).

Safety Precautions

- Not for use in humans and animals. For research purposes only.
- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards. Do not pipette by mouth.
- The preparation of the reagents requires the addition of formamide. Please consider all the safety precautions related to the storage, handling and waste disposal of formamide and solutions containing formamide.
- Step 5.3.3. f this protocol requires the addition of formaldehyde. Please consider all the safety precautions related to the storage, handling and waste disposal of formaldehyde and solutions containing formaldehyde.
- Discard all waste associated with specimens in a biohazard waste container.
- Please refer to the corresponding MSDS.

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. Fisherbrand™ Superfrost™ Plus Microscope Slides, Cat. No. 22-037-246
- Slide rack, e.g. Dutscher, Cat. HS15981A
- Ethanol
- Formaldehyde (CAS 50-00-0) 37% (v/v)
- Formamide (CAS 75-12-7) 100%
- Pepsin (CAS 9001-75-6)
- Heating block adjustable to 80°C, e.g. Stuart SD160, or Leica Thermobrite for slides
- Glass Hellendahl jars wrapped with alufoil (for the photosensitive steps) or non-transparent porcelain jars, 100 mL, e.g. Dutscher, Cat. No. BPL018
- Glass coverslips, 24×24 mm or 24 x 50 mm, e.g. Merck Art.No. C8181
- Fluorescence microscopy equipped with filters for FITC or Cy3 and DAPI, and with objectives with at least 40x magnification
- Sterile glass bottles with appropriate volume
- Pipettors, serological pipettors and pipette tips of appropriate volume to measure and transfer liquids between source containers and target sites
- Autoclaved 1.5 mL tubes
- Forceps
- Paper tissues

3. Assay Description

Note 3

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. Slides are spread and stored at –20°C until use according to the conventional protocols[1][3]. 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 and telomere 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 micronuclei as well as the centromere and telomere regions of chromosomes.

The FITC- labeled centromere probe and the Cy3-labeled telomere probe is added to the slide, denatured, and hybridized to their target 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 [2].

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].

The technology is compatible with the automated slide processing and image analysis system provided by MetaSystems [4].

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

- Biological dosimetry [12]
- 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]

If the aim of the experiment is to assess the genotoxic potential of a chemical, an additional negative control should be added, in which the solvent carrying the chemical is tested on its own to establish a baseline frequency of micronucleus formation and other chromosomal aberration-related events without the presence of the test chemical.

Cytochalasin B treatment is recommended in order to have cells completing mitosis during or after 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]. Studies in the literature report S9 in various percentages, for example 2% rat liver S9 with TK6 cells [8].

Note 4

Please note that the S9 fraction and the S9 cofactor solution kit are not included in this kit but are available separately from Xenometrix (Phenobarbital/β-Naphthoflavone induced frozen liquid rat liver S9, 1 mL, art.no. PRS-PB01LIQ, or 5 mL, art.no. PRS-PB05LIQ and S9 Cofactor Kit, 25 mL, art.no. PCO-0800).

Note 5

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 require 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)

Note 6

The Rapid MicroNucleus MoA Centromere Telomere kit can be used on any human cell but **not** on animal cells. The telomere probes can hybridize with the telomere region of mouse chromosomes, but it results in higher background signal, therefore the application of the Kit must not be applied on animal cells or animal derived cell lines.

The following cell lines / cell cultures can be used in accordance with OECD TG 473 [3]:

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

The following cell lines / cell cultures can be used in accordance with OECD TG 487 [1]:

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

The following cell lines can be used in accordance with OECD TG 487 [1]:

- HT-29 human colorectal adenocarcinoma cell line
- Caco-2 human colorectal adenocarcinoma cell line
- HepaRG human bipotent liver progenitor cell line
- HepG2 human hepatocellular carcinoma cell line
- A549 adenocarcinomic human alveolar basal epithelial cell line

4. Introduction

The Rapid MicroNucleus MoA Centromere Telomere Kit permits to identify directly clastogenic and aneugenic compounds in addition to the scoring of micronucleus in line with TG OECD 487.[1] Rapid MicroNucleus MoA Centromere Telomere is designed for an easier and more reliable detection of all human centromere and telomere sequences with high efficiency and strong signal.

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-centromere and telomere probes was proposed to discriminate not only clastogenic effect (MN without centromere and / or telomere staining) to aneugenic effects (MN with centromere and telomere staining) (Figures 1–3), but also to increase the sensitivity of this technique.[2]

Rapid MicroNucleus MoA Centromere Telomere provides in one step:

- easy and reliable detection of micronuclei
- direct identification of castogenic and aneugenic compounds, i.e. information on the mode of action, by which the test chemical exerts its genotoxic effect:

Rapid MicroNucleus MoA Centromere Telomere reduces the analysis time while considerably improving its precision compared to conventional and molecular techniques (2 h vs. 16 h).

Rapid MicroNucleus MoA Centromere Telomere shows a high specificity, there are no false positive results due to interferences by RNA, low-density micronuclei, precipitates, or cytoplasmic alterations.

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

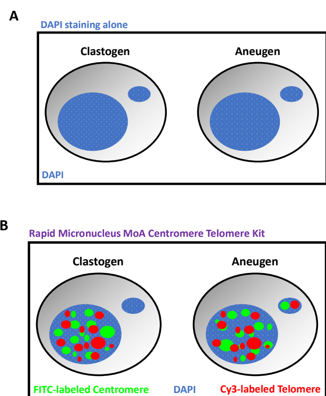


Figure 1. Schematic representation of the readout of the Rapid MicroNucleus MoA Centromere Telomere kit. DAPI staining alone cannot discriminate whether the Mode of Action of the genotoxic agent is aneugenic or clastogenic (Figure 1A), whereas centromere and telomere staining in addition to DAPI staining can easily detect MN with centromere / telomere sequences - aneugenic effect and can discriminate MN without centromere / telomere signal - clastogenic effect (Figure 1B).

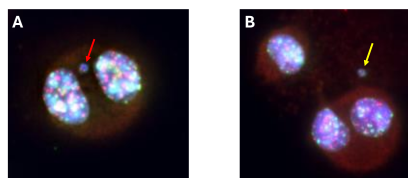
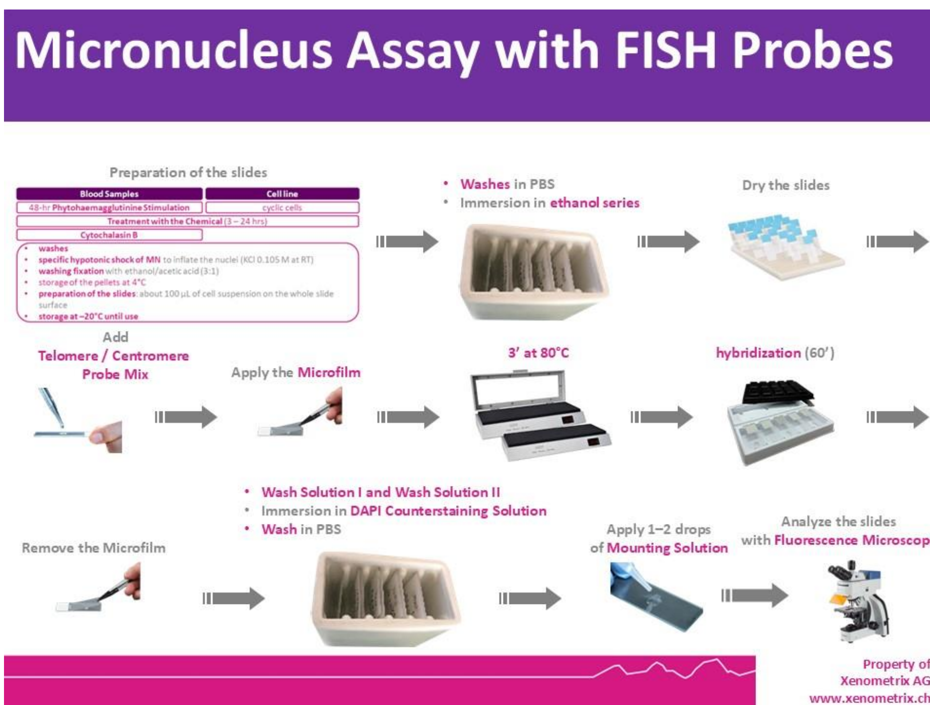


Figure 2. Binucleated cells obtained via the CBNM assay and stained with the Rapid MicroNucleus MoA Centromere Telomere kit. Centromere sequences (green signal) and telomere sequences (red signal) were stained and nuclei and micronuclei were counterstained using DAPI (blue signal).
A) Red arrow points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

5. Assay Procedure after Treatment of Cells (blood cultures, cell lines)



Related Xenometrix Products

Art. Nr.	Product	Details
KXT 96.1200	Kit: XTT Cytotoxicity test	1200 tests
PRS-PB01LIQ PRS-PB05LIQ	Post-Mitochondrial S9 Fraction, Phenobarbital-5,6 Benzoflavone induced, liquid, frozen Post-Mitochondrial S9 Fraction, Phenobarbital-5,6 Benzoflavone induced, liquid, frozen	1 mL 5 mL
PCO-0800	S9 Cofactor Solution for S9 Mix	25 mL

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