IVD CE 2797 UKCA

Instruction For Use

For Professional Use Only

MetaSystems Probes

Further information and other languages available at www.metasystems-probes.com
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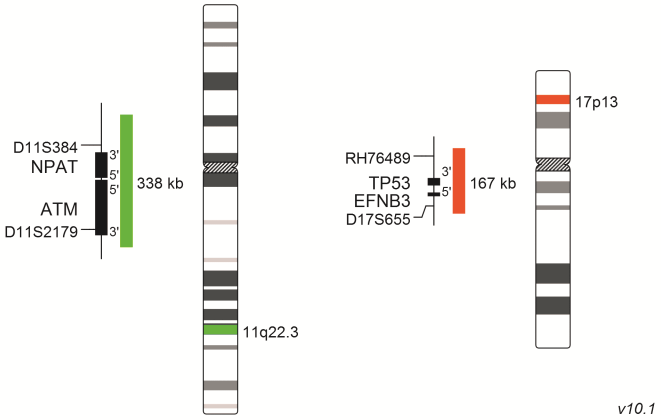
Product	Label	Reference No.	UDI-DI	Pack Size
XL ATM/TP53	green/orange	D-5046-100-OG	04251315812062	100 µl



Intended Purpose

The XL ATM/TP53 probe is a qualitative, non-automated test for the detection of deletions of the *ATM* gene region at 11q22.3 and the *TP53* gene region at 17p13 by fluorescence in situ hybridization (FISH). The product is intended as a diagnostic aid and assists in disease monitoring. The test population for the detection of deletions of the *ATM* gene region at 11q22.3 consists of patients with confirmed or suspected chronic lymphocytic leukemia (CLL), T-cell prolymphocytic leukemia (T-PLL) and non-Hodgkin-lymphomas (NHL). The test population for the detection of deletions of the TP53 gene region consists of patients with confirmed or suspected acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), myelodysplastic neoplasms (MDS), multiple myeloma (MM) and non-Hodgkin-lymphomas (NHL). Hybridization is to be performed on methanol/acetic-acid fixed cells derived from bone marrow or peripheral blood for ALL, AML, CLL, T-PLL and MDS, on methanol/acetic-acid fixed plasma cells for MM and on methanol/acetic-acid fixed cells derived from involved lymph node, involved bone marrow or other involved tissue for NHL.

Product Description

The XL ATM/TP53 probe consists of a green-labeled probe hybridizing to the *ATM* gene region at 11q22.3 and an orange-labeled probe hybridizing to the *TP53* gene region at 17p13.

Known Cross-Reactivity

No known cross-hybridizations.

Materials Provided

100 µl (10 tests) of XL ATM/TP53, the probe is dissolved in hybridization solution (> 50 % v/v formamide, < 30 % w/v dextran sulfate and < 2x SSC (saline-sodium citrate)) and ready to use.

Limitations

Analysis, data interpretation, and reporting of diagnostic findings received by FISH should be performed in accordance with professional norms and relevant guidelines by qualified and experienced personnel. The product is not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic. A diagnostic investigation obtained by this product is therefore always made in conjunction with other diagnostic methods. Deviations from the manufacturer's protocols may affect the robustness and performance of the test and may result in misleading findings. Probe maps are created in accordance with the intended purpose of the product. Solid colored bars do not necessarily mean that the probe completely covers the indicated genomic region. Only gaps larger than 10 kb are shown. Therefore, caution is advised when interpreting results generated through off-label use. Further information is available on request.

As a German based IVD manufacturer and distributor, MetaSystems Probes follows current European and German regulations, which prohibit any statement regarding interpretation of patient-related data, as well as diagnostic and therapeutic recommendations, respectively.

Storage and Handling

Probes should be stored in the dark at -20 °C (±5 °C). Probe performance has been shown to be unaffected for up to 20 freeze-thaw cycles. Probes are light sensitive, therefore, exposure to light should be limited to a minimum during handling.

Shipping

Products produced by MetaSystems Probes are shipped at room temperature.

Equipment and Materials Necessary but not Supplied

- Water bath with accurate temperature control
- Hotplate, with solid plate and accurate temperature control
- Micro-pipettes with volumes ranging from 1 µl to 1 ml, calibrated
- pH meter, calibrated
- Freezer -20 °C (±5 °C)
- Humidified chamber 37 °C (± 1 °C)
- Fluorescence microscope with suitable filters (see below)
- Immersion oil, recommended by the microscope manufacturer (fluorescence grade)
- Thermometer
- DAPI/antifade
- Rubber cement
- Microscope slides
- Coverslips (glass): 22 mm x 22 mm and 24 mm x 32 mm
- Gloves
- Coplin jars
- Forceps
- 20x SSC
- Tween-20
- Distilled water
- Microcentrifuge
- Timer

Fluorochrome Specification

Label	Excitation max.	Emission max.
Green	505 nm	530 nm
Orange	552 nm	576 nm

Fluorescence Microscope Recommendation

- Fluorescence Illumination: Suitable metal halide or LED fluorescence illumination systems or conventional 100 watt mercury lamp illuminators.
- Objectives suitable for epi-fluorescent illumination.
- Fluorescence Filters: For viewing/counting use an appropriate multi bandpass filter set. For capturing images or observing individual color channels on the microscope, use suitable single bandpass filter sets for the respective fluorochromes (e.g., from MetaSystems).

Test Principle

Fluorescence in situ hybridization (FISH) uses DNA fragments in which fluorophore-coupled nucleotides are incorporated to detect complementary DNA sequences in fixed cells under a fluorescence microscope. The DNA of the selected probes and the patient DNA are denatured, meaning that the two DNA strands of the double helix are separated. During subsequent renaturation, the DNA probes hybridize to complementary sequences of the patient DNA.

Sample Preparation

General Comments

The product is designed for use as specified in the intended purpose. Methanol/acetic acid fixed cells are prepared from bone marrow aspirates, peripheral blood or released single cells from involved lymph nodes after careful mincing of the nodal tissue. For multiple myeloma it is recommended to enrich CD138+ plasma cells (e.g., magnetic cell sorting) or identify them in situ with an appropriate fluorescently labeled antibody (clg-FISH).
Further details on standard procedures can be found in laboratory manuals and publications (e.g., The AGT Cytogenetics Laboratory Manual, 4th Edition, for lymphoma see also Campbell et al,(2015) Pathology 37:493-507 and Ross (2004) Curr Diagn Pathol 10:345-350, for CD138+ cell enrichment see also Cumova et al (2010) Int J Hematol 92:314-319 and for clg-FISH see also Gole et al (2012) Blood 120:4792.

Stability of Hybridized Slides

- Hybridized FISH slides can be analyzed for at least six months if stored in the dark at -20 °C (±5 °C).

Additional Process Recommendations

- The use of a calibrated thermometer is strongly recommended for measuring temperatures of solutions, water baths, and incubators, as these temperatures are critical for optimal product performance.
- Carefully check the temperature of preheated solutions.
- Carefully check the pH value of all solutions. It must be in the range of 7.0 - 7.5 at room temperature.
- Concentrations of buffers (stringency), pH and temperature are important because low stringency can lead to non-specific binding of the probe and too high stringency can lead to reduced signal intensity or vanished signals.
- Before opening: spin briefly to collect probe at the bottom of the tube.

FISH Protocol for MetaSystems' DNA Probes

Slide Preparation

1. Drop cell sample onto microscope slide. Allow to air dry. If the slides are not used within the next couple of days, store at -20 °C (±5 °C).
2. Apply 10 µl of probe.
3. Cover with coverslip 22 mm x 22 mm.
4. Seal with rubber cement.

Denaturation

1. Denature sample and probe simultaneously by heating slide on a hotplate at 75 °C (±1 °C) for 2 min.

Hybridization

1. Incubate in a humidified chamber at 37 °C (±1 °C) overnight.

Post-Hybridization Washes

Solutions Required

- 0.4x SSC (pH 7.0 – 7.5) at 72 °C (±1 °C)
- 2x SSC, 0.05 % Tween-20 (pH 7.0) at room temperature

Procedure

1. Remove coverslip and all traces of glue carefully.
2. Wash slide in 0.4x SSC (pH 7.0) at 72 °C (±1 °C) for 2 min.
3. Drain slide and wash in 2x SSC, 0.05 % Tween-20 (pH 7.0) at room temperature for 30 seconds.
4. Rinse briefly in distilled water to avoid crystal formation and let air dry.

Counterstain

Solutions required:

- DAPI/antifade (e.g., MetaSystems Probes DAPI/antifade, D-0902-500-DA)

Procedure:

1. Apply 10 µl of the DAPI/antifade and overlay with a 24 mm x 32 mm coverslip.
2. Allow the penetration of DAPI/antifade for 10 min.
3. Proceed with microscopy and analysis.
4. Store slides at –20 °C (±5 °C).

Expected Results

Only the most frequent signal constellations are shown, other relevant signal patterns may be observed.

Normal Cell:
Two green (2G) and two orange (2O) signals.

Aberrant Cell (typical results):
One green (1G) and two orange (2O) signal resulting from loss of one green signal.

Aberrant Cell (typical results):
Two green (2G) and one orange (1O) signal resulting from loss of one orange signal.

Analytical Performance

Analytical performance data were collected using interphase nuclei from PHA-stimulated lymphocyte cultures. Cell harvesting was performed according to cytogenetic standards. Equivalence of results between unstimulated bone marrow cultures and PHA-stimulated lymphocytes was ensured by a comparative study.

Analytical Specificity

The specificity is calculated as the percentage of correct targets detected out of the total number of targets detected.

The calculated analytical specificity is 100 % after 20 evaluated metaphases from 5 different karyotypically normal males.

Analytical Sensitivity

Analytical sensitivity is calculated as the percentage of interphase nuclei that have the expected normal signal pattern out of the total number of interphase nuclei analyzed. The signal pattern of 400 nuclei from each of 10 karyotypically normal individuals were analyzed.

The degree of deviation from the mean is represented by the relative standard deviation (% RSD).

Pattern	Sensitivity	% RSD
2G 2O (normal)	97.1 %	1.3 %

Cut-Off

The cut-off for a qualitative test is the threshold above which the result is considered positive and below which the result is considered negative.

The cut-off value was calculated based on probe hybridizations on interphase nuclei of 10 karyotypically normal individuals. Cut-off values are based on 400 scored nuclei each.

Pattern	Cut-Off
1G 2O	3.9 %
2G 1O	5.1 %

The cut-off value is informative and depends on several laboratory-related parameters. Therefore, for diagnostic use, cut-off values have to be determined individually by each laboratory.

Precision (Reproducibility/ Repeatability)

Reproducibility is the degree of agreement between the results of analytical sensitivity studies conducted under different conditions (day, lot and sample). For each condition three analyses were performed with 100 nuclei each.

Reproducibility is given as the degree of deviation from the mean by the relative standard deviation (% RSD).

Conditions	Reproducibility % RSD
Day-to-Day same lot and same individual on three days	2.7 %
Lot-to-Lot same individual and day with three lots	3.2 %
Sample-to-Sample same lot and day with three individuals	0 %

Repeatability is the degree of agreement between studies conducted under same conditions. Separate repeatability studies were not performed as the degree of deviation from the mean in reproducibility studies under different conditions was determined with ≤5 % relative standard deviation (% RSD). Hence, a degree of deviation from the mean under equal conditions with ≤5 % relative standard deviation (% RSD) was concluded.

Clinical Performance

Published Experience Gained by Routine Diagnostic Testing

Data from routine diagnostic testing were obtained from European diagnostic laboratories and published on the MetaSystems Probes website (see respective performance data sheet in the corresponding download section for this specific product). The presence of the target marker in positive cases and the absence in negative cases detected by FISH was confirmed by reference technologies (chromosome banding analysis and whole genome sequencing). The test cohort includes patients with confirmed or suspected AML, ALL, CLL, MDS, MPN and NHL. Specimens were obtained from bone marrow and peripheral blood.

Rearrangement	No. of Cases	Diagnostic Sensitivity	Diagnostic Specificity
Deletions of the *ATM* gene region at 11q22.3	544	96.2 %	99.7 %
Deletions of the *TP53* gene region at 17p13	544	95.4 %	99.6 %

Other Sources of Clinical Performance Data

Evaluation of the diagnostic validation data collected as part of the IVDD and classified as other sources of clinical performance data shows that XL ATM/TP53 correctly identified all 9 aberrant cases analyzed.

Quality Control Procedure

Before initial use of this product in diagnostics, it should be verified that it performs as expected. Guidance and recommendations for implementation of new FISH tests in diagnostics must be considered (e.g., CLSI Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline – Second Edition).

Safety Instructions

All probes produced by MetaSystems Probes are for professional use only and should be used by qualified and trained personnel. To ensure safe operation and reproducible results please observe the safety notices and caution signs below.

DANGER

Contains:	Formamide
Hazard statements:	H360FD May damage fertility. May damage the unborn child. H351 Suspected of causing cancer. H373 May cause damage to organs through prolonged or repeated exposure.
Precaution statements:	P201 Obtain special instructions before use. P260 Do not breathe dust/fume/gas/mist/vapors/spray. P280 Wear protective gloves/protective clothing. P308+ P313 IF exposed or concerned: Get medical advice/attention. P501 Dispose of contents/container to in accordance with local/regional/national/international regulations.
Special labeling:	Restricted to professional users.

CAUTION: Hot water bath and hot plates!
For denaturation and hybridization hot water baths and hot plates are used with temperatures of >37 °C. Be careful not to get in direct contact with hot surfaces or liquids.
Wear gloves and a lab coat. In case of contact with skin, cool immediately with cold water.

ATTENTION: Good Laboratory Practice!
Use in accordance with the principles of good laboratory practice.

Adverse Event Reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member state in which the user and/or the patient is established.

Troubleshooting

Problem	Potential Cause(s)	Recommended Solution
No FISH signals are detected in the microscope.	• Reflected light shutter closed / stop slider in light path.	• Open shutter / move stop slider out of the light path.
	• Fluorescent lamp is switched off.	• Switch on fluorescent lamp.
	• Wrong fluorescence filter is in light path.	• Move correct filter into light path.
	• Objective is out of position.	• Swing objective into light path.
	• Phototube is in camera position.	• Direct light path to eyepieces.
Hybridization signals become weak after a while.	• The immersion oil has penetrated between the glass slide and cover glass.	• Replace coverslip and DAPI/antifade. Use 24 mm x 32 mm coverslip even if only a small region is hybridized.
Diffuse signals.	• Preparation is not adequately illuminated.	• Check optical pathway of microscope. Adjust the UV light properly. Check the lifetime of the UV lamp.
	• Focus plane cannot be adjusted properly.	• Use sufficient immersion oil. Do not mix different immersion oils. Use immersion oil suitable for fluorescence. • Antifade layer is too thick for focusing. Do not use too much DAPI/antifade. 10 µl per slide (24 mm x 32 mm coverslip) are sufficient. • Use suitable coverslips.
Weak signals.	• Slide preparation is too old.	• Slides should not be older than two weeks. If the slides are not used within this period, store at -20 °C (±5 °C)
	• Denaturation is not adequate.	• Aging, baking, or further fixation may inhibit the hybridization and is not recommended. • Increase denaturation temperature up to 80 °C or increase denaturation time to 3 min.
	• A multi bandpass filter is used for viewing.	• Use a dedicated single bandpass filter.
Weak aqua or green signals or high diffuse background in green color channel.	• DAPI intensity is too high resulting in crosstalk to AQUA filter or GREEN filter.	• Use DAPI/antifade of low concentration.
	• pH value of washing solutions is too low.	• Ensure that pH value is between 7.0 and 7.5 of solutions. Some green fluorophores are very sensitive to pH below 7.
High unspecific background.	• Remaining cytoplasmic proteins of the cells may impair the hybridization.	• Pretreat slides with Pepsin.
If the recommended measures do not solve the problem, or your problem is not listed, please contact MetaSystems Probes.		

Customer Support

Please contact MetaSystems Probes GmbH (contact details see below) or our authorized distributor in your country.

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For *Summary of Safety and Performance* visit website https://ec.europa.eu/tools/eudamed/#/screen/home or inquire at info@metasystems-probes.com.

Symbols Used

Symbol	Description
IVD	Indicates a *medical device* that is intended to be used as an in vitro diagnostic *medical device.*
CE	Conformity mark that indicates that a device is in conformity with the applicable regulatory requirements in the European Union.
UK CA	Conformity mark that indicates that a device is in conformity with the applicable regulatory requirements in Great Britain.
	Indicates the *medical device manufacturer.*
	Indicates that caution is necessary when operating the device or control close to where the *symbol* is placed, or that the current situation needs operator awareness or operator action in order to avoid undesirable consequences.
	Indicates the need for the user to consult the *instructions for use*.

Document Revision

Revision	Issue Date	Indication for Change
CE-IVD-RevA230926-231218v10.1	18.12.2023	Updated IFU according to REGULATION (EU) 2017/746 on in vitro diagnostic medical devices. The update does not affect device characteristics directly and does not have impact on the composition or ingredients of our products. It does also not influence the way our products are applied, but triggers changes to the information provided with the product as labels, intended purpose and instructions for use.