A Sysmex Group Company

CytoCell

REF: RU-PMP 025 / RU-PMP 026 / RU-PMP 027 / RU-PMP 028

Multiprobe AML/MDS Panel (RUO)

Research Use Only

PROFESSIONAL USE ONLY

ENGLISH

Further information available at www.ogt.com

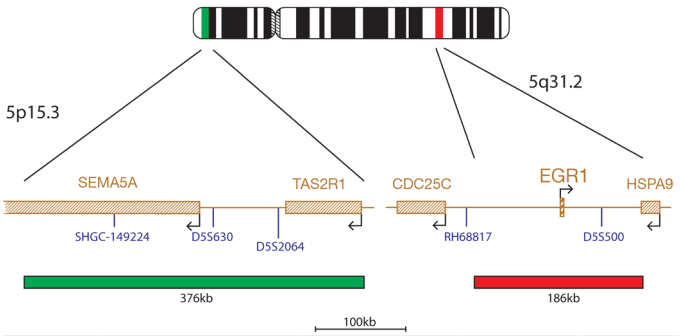
To maintain optimum storage conditions, when not in use ensure the kit is stored in the additional zip-lock bag provided. Ensure the silica gel remains in the kit box at all times and is not discarded.

Fluorescence *in situ* hybridisation (FISH) is a technique that allows DNA sequences to be detected on metaphase chromosomes or in interphase nuclei from fixed cytogenetic samples. The technique uses DNA probes that hybridise to entire chromosomes or single unique sequences, and serves as a powerful adjunct to classic cytogenetics. Target DNA, after fixation and denaturation, is available for annealing to a similarly denatured, fluorescently labelled DNA probe, which has a complementary sequence. Following hybridisation, unbound and non-specifically bound DNA probe is removed and the DNA is counterstained for visualisation. Fluorescence microscopy then allows the visualisation of the hybridised probe on the target material.

Intended Use
This product is intended to be used for research use only and is not for use in diagnostic procedures.

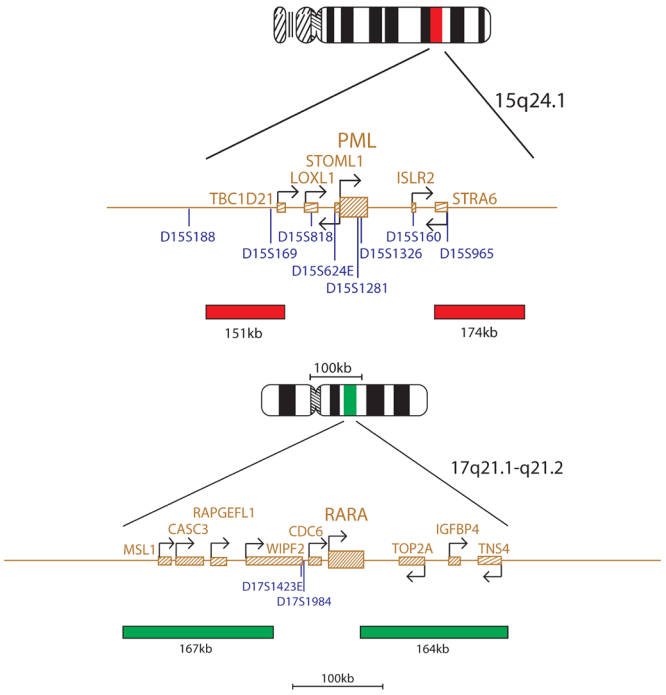
Probe Specification

Del(5q) Deletion
EGR1, 5q31.2, Red
5p15.3, Green



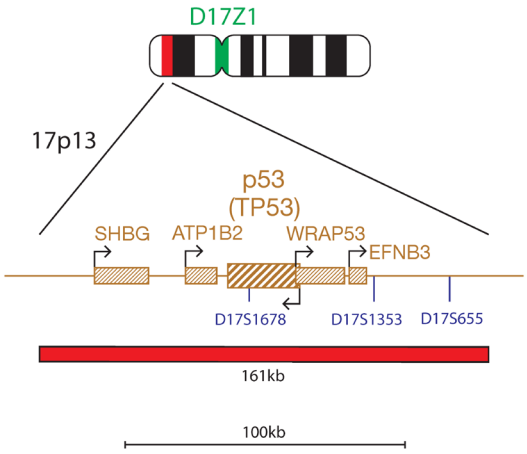
The EGR1 probe, labelled in red, covers a 186kb region within 5q31.2, including the D5S500 marker. The probe mix also contains a control probe for chromosome 5 at 5p15.3, including the marker D5S630, labelled in green.

PML/RARα Translocation, Dual Fusion
PML, 15q24.1, Red
RARα, 17q21.1-q21.2, Green



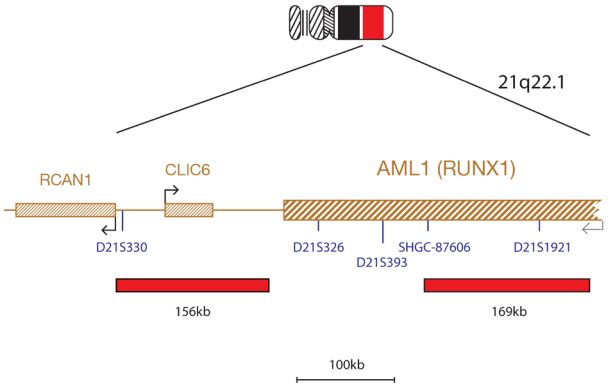
The PML probe mix, labelled in red, consists of a 151kb probe centromeric to the PML gene and a 174kb probe telomeric to the PML gene. The RARα probe mix, labelled in green, consists of a 167kb probe centromeric to the RARα (RARA) gene, including the CASC3 gene, and a 164kb probe, including the telomeric end of the RARα gene as well as the TOP2A and IGFBP4 genes.

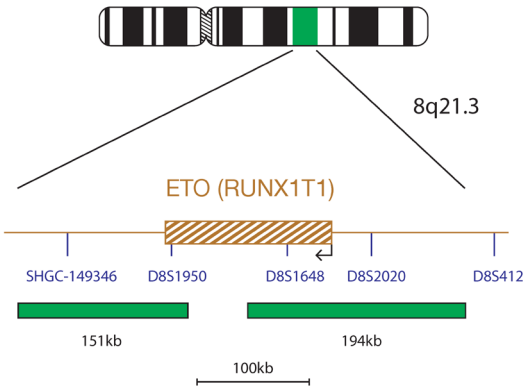
P53 Deletion
P53, 17p13, Red
D17Z1, 17p11.1-q11.1, Green



The P53 probe is 161kb in length and is labelled in red. It covers the whole P53 (TP53) gene and flanking regions. The probe mix also contains a control probe for the 17 centromere (D17Z1) labelled in green.

AML1/ETO Translocation, Dual Fusion
AML1, 21q22.1, Red
ETO, 8q21.3, Green

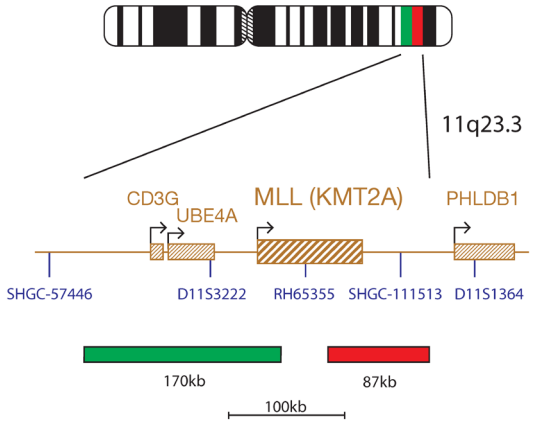




The AML1 probe mix, labelled in red, consists of a 156kb probe centromeric to the AML1 (RUNX1) gene, including the CLIC6 gene and a 169kb probe covering part of the AML1 (RUNX1) gene and extending beyond the marker D21S1921. The ETO probe mix, labelled in green, consists of two probes, 151kb and 194kb, on either side of the ETO (RUNX1T1) gene.

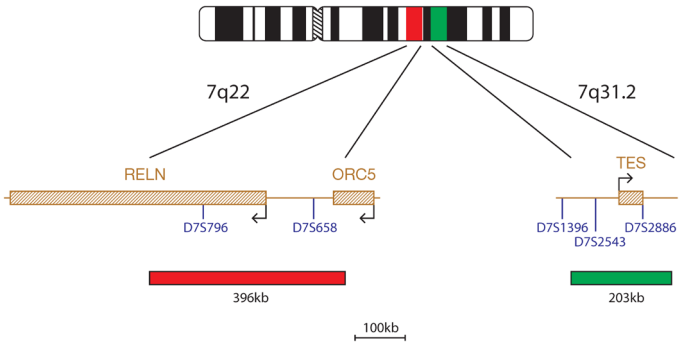
Chromosome 8
The Multiprobe AML/MDS Panel (RUO) has been designed so that chromosome 8 can be enumerated using the ETO probe on square 4.

MLL Breakapart
MLL, 11q23.3, Red
MLL, 11q23.3, Green



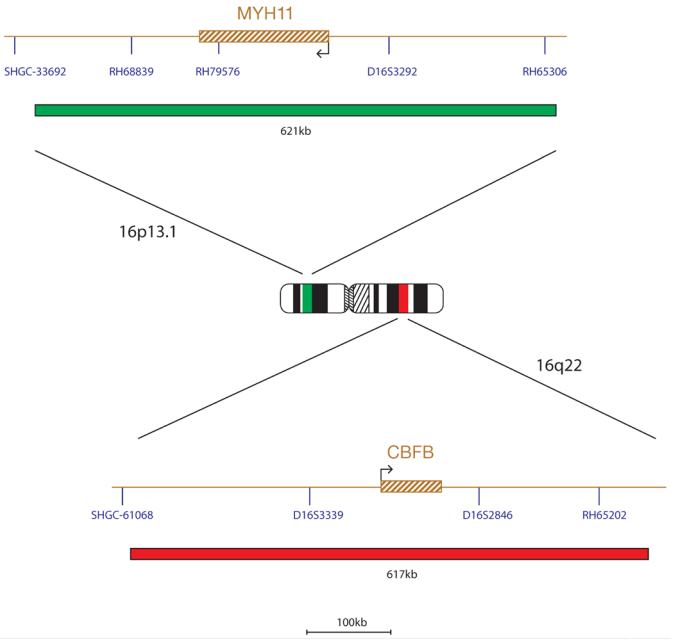
The MLL product consists of an 87kb probe, labelled in red, covering a region telomeric to the MLL (KMT2A) gene including the marker SHGC-111513 and a green probe covering a 170kb region centromeric to the MLL gene spanning the CD3G and UBE4A genes.

Del(7q) Deletion
7q22. Red
7q31.2, Green



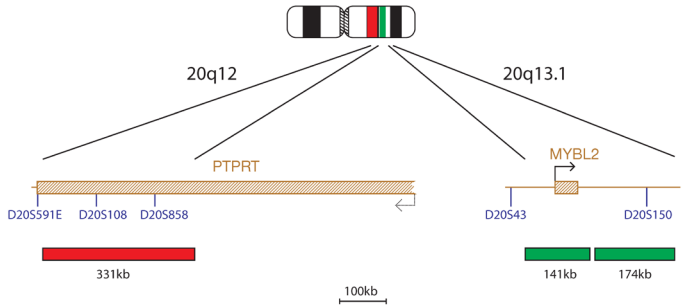
The 7q22 probe, labelled in red, covers a 396kb region including the telomeric end of the RELN gene and extending beyond the marker D7S658. The 7q31.2 probe, labelled in green, covers a 203kb region including the TES gene.

CBFβ/MYH11 Translocation, Dual Fusion
CBFβ, 16q22, Red
MYH11, 16p13.1, Green



The CBFβ probe, labelled in red, covers a 617kb region within 16q22 and includes the CBFβ gene. The MYH11 probe, labelled in green, covers a 621kb region within 16p13.1 and includes the MYH11 gene.

Del(20q) Deletion
20q12, Red
20q13.1, Green



The 20q12 probe, labelled in red, covers a 331kb region within the PTPRT gene and includes the D20S108 marker. The 20q13.1 probes, labelled in green (141kb and 174kb), cover the MYBL2 gene and include the D20S150 marker.

Materials Provided
Each kit contains the following reagents, which are sufficient for either 2 (RU-PMP 025), 5 (RU-PMP 026) or 10 (RU-PMP 027) devices*:

1. 2, 5 or 10 Multiprobe AML/MDS Panel (RUO) devices coated with directly labelled probes.
2. 4, 7 or 12 glass slides printed with a special template.
3. 500µl Hybridisation Solution (Formamide, Dextran Sulphate, SSC).
4. 1 CytoCell Slide Surface Thermometer.
5. 1 CytoCell Hybridisation Chamber.

*20 device kit (RU-PMP 028) is supplied as 4x5 Multiprobe AML/MDS Panel (RUO) devices.

Warnings and Precautions

1. For research use only. Not for use in diagnostic procedures. For laboratory professional use only.
2. Wear gloves when handling Hybridisation solution, DNA probes and DAPI counterstain.
3. Hybridisation Solution contains formamide, which is a teratogen; do not breathe fumes or allow skin contact. Handle with care; wear gloves and a lab coat.
4. DAPI is a potential carcinogen. Handle with care; wear gloves and a lab coat.
5. Follow local disposal regulations for your location along with recommendations in the Safety Data Sheet to determine the safe disposal of this product.
6. Operators must be capable of visually distinguishing between red, blue and green.
7. Room temperature (RT) = 15ºC to 25ºC

Storage and Handling
The Multiprobe AML/MDS Panel (RUO) kit should be stored at 2-8ºC. **Do not freeze**. When not in use ensure the kit is stored in the additional zip-lock bag provided. Ensure the silica gel remains in the kit box at all times and is not discarded.

Based on the stability established for CytoCell Multiprobes, this product should be stable for 3 months post manufacture date, when stored as indicated on the label and in these instructions for use.

Protocol Recommendations

Equipment and Materials Necessary but not Supplied

1. 500µl DAPI Antifade ES counterstain (0.125µg/ml DAPI (4,6-diamidino-2-phenylindole) in glycerol-based mounting medium).
2. Hotplate (with a solid plate and accurate temperature control up to 80ºC).
3. Variable volume micropipettes and tips range 1µl - 200µl.
4. Water bath with accurate temperature control at 72ºC.
5. Microcentrifuge tubes (0.5ml).
6. Fluorescence microscope (Please see Fluorescence Microscope Recommendation section).
7. Plastic or glass coplin jars.
8. Forceps.
9. Fluorescence grade microscope lens immersion oil.
10. Bench top centrifuge.
11. Microscope slides.
12. Timer.
13. 37ºC water bath without stirrer.

Fluorescence Microscope Recommendation

Use a 100-watt mercury lamp and plan apochromat objectives x63 or x100 for optimal visualisation. Use a triple bandpass filter DAPI/FITC/Texas Red for optimal visualisation of the green and red fluorophores and DAPI simultaneously.
Check the fluorescence microscope before use to ensure it is operating correctly. Use immersion oil that is suitable for fluorescence microscopy and formulated for low autofluorescence. Avoid mixing DAPI Antifade with microscope immersion oil as this will obscure signals. Follow manufacturers' recommendations in regards to the life of the lamp and the age of the filters.

Sample Preparation

Samples should be prepared according to the laboratory or institution guidelines. Prepare air-dried samples on CytoCell Multiprobe AML/MDS Panel (RUO) template slides according to CytoCell's protocol below. Baking or otherwise ageing slides is not recommended as it may reduce signal fluorescence.

Multiprobe AML/MDS Panel (RUO) Protocol

Please note: The probes used on the Multiprobe AML/MDS Panel (RUO) device are directly labelled with fluorophores, which are light sensitive. Ensure that exposure of the probes to laboratory lights is limited at all times (it is not necessary to work in the dark).

1. Slide preparation

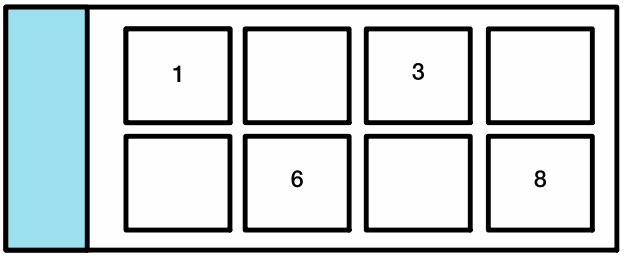
a) Clean a template slide. Soak the template slide for 2 minutes in 100% methanol and polish dry with a clean soft tissue.

b) Establish the correct mitotic index. It is important that the intended sample has a sufficiently high mitotic index to allow analysis of the sample. To check the density of the sample, using a micropipette (e.g. a Gilson P10 or P20) pipette 4µl of the cell suspension onto one of the areas of the spare template slide and allow to air dry. The small volume used means that you usually have to gently touch the slide with the pipette tip to transfer the suspension. Examine by phase contrast microscopy. If the cell density is too high, dilute the suspension with fresh fixative. If the mitotic index is too low, spin down the fixed cell suspension at 160xg for 10 minutes. Note the volume of supernatant, remove, and re-suspend the cell pellet in a smaller volume of fresh fixative. If cell sample density has been altered, spot 4µl of the concentrated sample onto another square of your test slide and re-examine by phase contrast microscopy.

Please Note: 50µl is the minimum volume required for the protocol.

c) Quality control of samples. Samples should be examined for cytoplasm since this will interfere with the *in situ* protocol. If the chromosomes appear to be enclosed by a granular material when examined under phase contrast microscopy, then this will compromise results. One method for reducing cytoplasm is to spot 4µl of your sample onto the template slide and watch the fixative as it spreads out: in the normal situation, the fixative will spread to maximum, recede and then evaporate. To clean up any cytoplasm we have found that effective results are achieved if a fresh drop of fixative is allowed to fall onto the spot at the point when the spreading fixative has reached its maximum. Allow the drop of fixative to evaporate and re-examine the spot.

d) Spotting of the slide. Pipette 4µl of cell suspension onto all 8 areas of the template slide in a sequence of alternating squares as shown below. This will prevent the cell spreads from interfering with each other.



e) Once the first group of drops has air-dried, spot the remaining squares with 4µl drops in the same manner. After the slide has dried, examination of the slide under phase contrast will reveal whether any squares have been missed. If spots have been missed, or squares have too few cells, simply spot those squares again: it is not necessary to re-spot a new slide. If upon examination of the slide, a square has insufficient cells/metaphases, further drop(s) of suspension can be added to increase the cell density.

Please note: If the metaphases appear overspread, then clean a new template slide thoroughly in methanol and re-spot allowing every spot to dry before proceeding to the next.

2. Preparation of the Multiprobe AML/MDS Panel (RUO) device and template slide

a) Ensure that the Hybridisation Chamber is in the 37ºC water bath and allow it to equilibrate to 37ºC (+/- 1ºC). This may take up to an hour if the water bath has been switched on from cold.

b) Mix the hybridisation solution by repeated pipetting and pre-warm a 25µl aliquot per device to 37ºC. Also pre-warm each device by placing it on a 37ºC hotplate, label side down. Do not touch the raised surfaces of the device.

c) Immerse template slides containing fixed samples in 2xSSC for 2 minutes at room temperature (RT) without agitation.

d) Whilst the device is still at 37ºC, dehydrate template slides containing fixed samples through an ethanol series (2 minutes each in 70%, 85% and 100%) at RT, air dry and place at 37ºC hotplate to warm up.

e) Add 2µl of pre-warmed hybridisation solution to each of the eight areas on the pre-warmed device using a P10 micropipette, while it remains on a 37ºC hotplate.

3. Positioning of the template slide over the device

a) Carefully invert the template slide over the device such that the number 1, which is now upside down, is located over the top right hand area of the device (Figure 1).

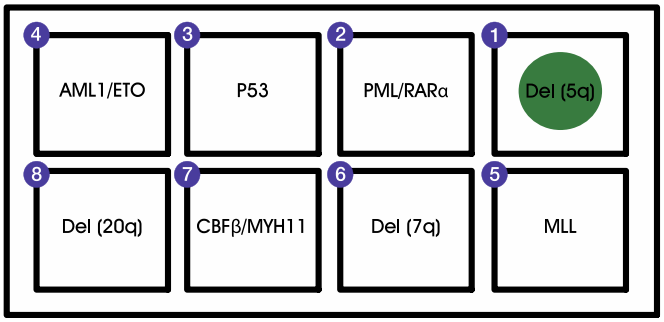


Figure 1. Location of the probes on the Multiprobe AML/MDS Panel (RUO). To help locate square 1, its position on the device has been marked with a coloured label.

b) Make sure that the template slide is carefully aligned with the matching areas on the device. Carefully lower the slide over the device so that the drops of hybridisation solution make contact with the slide. Apply gentle, even pressure to ensure that the hybridisation solution is spread to the edges of each of the raised areas on the device.

c) Lift the slide carefully holding the frosted end of the glass slide and invert so that the template slide is underneath the device. Make sure the device does not smear across the template slide as this could cause cross-contamination of the probes.

d) Place at 37ºC (+/- 1ºC) (hotplate or incubator) for 10 minutes.

4. Instructions for use of the CytoCell Slide Surface Thermometer

a) The temperature of the 75ºC hotplate should be checked with the CytoCell Slide Surface Thermometer before proceeding to denaturation.

b) To use the thermometer properly, place it onto the surface of the hotplate and wait until the different segments stop changing colour. The actual temperature is indicated by a deep aqua colour.

Please note:

c) When the segments appear granular and the colours no longer appear uniform and regular, the thermometer should be discarded as it is exhausted. The life span of each thermometer should, however, easily be sufficient for a ten-device kit.

d) This thermometer is a liquid crystal device and although reusable, it must be treated with care to ensure a reasonable life span. The thermometer must only be used to check the temperature of a hotplate; it must not be used to monitor the hotplate performance over time.

5. Denaturation

Please note: A PCR thermal cycler-heating block is NOT suitable for use in place of solid bed hotplate for this procedure.

a) Transfer the slide/device sandwich to the hotplate taking particular care to hold it level. Ensure the sample slide is in good contact with the hotplate.

b) Denature on the hotplate at 75ºC (+/- 1ºC) for 2 minutes.

6. Hybridisation

Place the slide/device sandwich in the pre-warmed Hybridisation Chamber, replace the lid and float the chamber in the 37ºC (+/- 1ºC) water bath (non-stirring) overnight.

Please note:

a) Do not seal the lid on the hybridisation chamber.
b) Do not place a lid on the water bath.
c) Do not hybridise in an incubator.
d) Please ensure that the hybridisation chamber is completely dry (i.e. no water or damp tissue inside the chamber).

The humidity inside the chamber is vital for optimal hybridisation. The correct levels will be achieved following those steps.

7. Post-hybridisation stringent washes

Please note: Avoid processing more than two slides through the stringency washes at any one time.

a) Remove the device carefully from the slide.
b) Immerse the slide in 0.4xSSC (pH 7.0) at 72ºC (+/- 1ºC) for 2 minutes without agitation.
c) Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.

8. Mounting and visualisation of results

a) Drain the slide and apply 20µl of DAPI antifade to each end of the slide.
b) Cover with a coverslip (24x50mm or 24x60mm), remove any bubbles and allow the colour to develop in the dark for 10 minutes.
c) View with a fluorescence microscope.

Please note: Certain types of microscope have slide holders, which make it difficult to view the extreme ends of the slide. If this occurs then simply turn the slide through 180°, which will help with the viewing of the slide.

Stability of Finished Slides

FISHed slides remain analysable for up to 1 month if stored in the dark at/or below RT.

Procedural Recommendations

1. Baking or ageing of slides may reduce signal fluorescence.
2. Hybridisation conditions may be adversely affected by the use of reagents other than those provided or recommended by Cytocell Ltd.
3. Use a calibrated thermometer for measuring temperatures of solutions, waterbaths and incubators as these temperatures are critical for optimum product performance.
4. The wash concentrations, pH and temperatures are important as low stringency can result in non-specific binding of the probe and too high stringency can result in a lack of signal.
5. Incomplete denaturation can result in lack of signal and over denaturation can also result in non-specific binding.
6. Over hybridisation can result in additional or unexpected signals.
7. Users should optimise the protocol for their own samples prior to use.
8. Suboptimal conditions may result in non-specific binding that may be misinterpreted as a probe signal.

Expected Results

Del(5q) Deletion

In a normal cell there should be two red and two green signals (2R, 2G). A cell with a hemizygous deletion of the 5q31.2 should have one red and two green signals (1R, 2G) whilst a cell with a homozygous deletion should have no red and two green signals (0R, 2G).

PML/RARα Translocation, Dual Fusion

In a normal cell these probes should appear as discrete red and green spots, one for each homologue (resulting in a 2R, 2G conformation). In a t(15;17)(q24.1;q21) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosomes 15 and 17 respectively (1R, 1G, 2Y).

P53 Deletion

In a normal cell there should be two red and two green signals (2R, 2G) whilst a cell with a P53 deletion should have one red and two green signals (1R, 2G).

AML1/ETO Translocation, Dual Fusion

In a normal cell these probes should appear as discrete red and green spots, one for each homologue (resulting in a 2R, 2G conformation). In a t(8;21)(q21.3;q22.12) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosomes 21 and 8 respectively (1R, 1G, 2Y).

MLL Breakapart

In a normal cell two red/green (or fused yellow) signals are expected (2Y). In a cell with MLL translocation there should be one distinct red and one green signal in addition to one red/green (or fused yellow) signal of the normal chromosome 11 (1R, 1G, 1Y).

Del(7q) Deletion

In a normal cell there should be two red and two green signals (2R, 2G). Deleted cells may show one of the following signal patterns:

1. If a deletion encompasses the proximal CDR only and is hemizygous one red and two green signals should be observed (1R, 2G).
2. If a deletion encompasses the proximal CDR only and is homozygous no red and two green signals should be observed (0R, 2G).
3. If a deletion encompasses the distal CDR only and is hemizygous two red and one green signals should be observed (2R, 1G)
4. If a deletion encompasses the distal CDR only and is homozygous two red and no green signals should be observed (2R, 0G)
5. One red and one green signal pattern (1R, 1G) can be observed in case of monosomy 7 or hemizygous deletion of both CDRs on 7q.

CBFβ/MYH11 Translocation, Dual Fusion

In a normal cell these probes should appear as discrete red and green spots, one for each homologue (resulting in a 2R, 2G conformation). In an Inv(16) cell or a t(16;16)(p13;q22) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosome 16 (1R, 1G, 2Y).

Del(20q) Deletion

In a normal cell there should be two red and two green signals (2R, 2G). Deleted cells may show one of the following signal patterns:

1. If a deletion is interstitial encompassing band q12 but not q13.12 and is hemizygous, one red and two green signals should be observed (1R, 2G).
2. If a deletion is interstitial encompassing band q12 but not q13.12 and is homozygous, no red and two green signals should be observed (0R, 2G).
3. If a deletion encompasses band q13.12 but not q12 and is hemizygous, two red and one green signals should be observed (2R, 1G).
4. If a deletion encompasses band q13.12 but not q12 and is homozygous, two red and no green signals should be observed (2R, 0G).
5. One red and one green signal pattern (1R, 1G) can be observed in case of monosomy 20 or hemizygous deletion of both bands on 20q.

Additional Information

For additional product information please contact the CytoCell Technical Support Department.
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Guide to Symbols

REF	**en:** Catalogue number
LOT	**en**: Batch code
	en: Consult instructions for use
	en: Manufacturer
	en: Use-by date
-15°C / -25°C	**en:** Temperature limit
	en: Keep away from sunlight
Σ	**en:** Contains sufficient for <n> tests
CONT	**en:** Contents

Patents and Trademarks

CytoCell is a registered trademark of Cytocell Ltd.

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