



A Sysmex Group Company



REF: RU-PMP 030 / RU-PMP 031 / RU-PMP 032 / RU-PMP 033

Multiprobe ALL Panel v2 (RUO)

Research Use Only

PROFESSIONAL USE ONLY

Further information available at www.cytocell.com

ENGLISH

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.

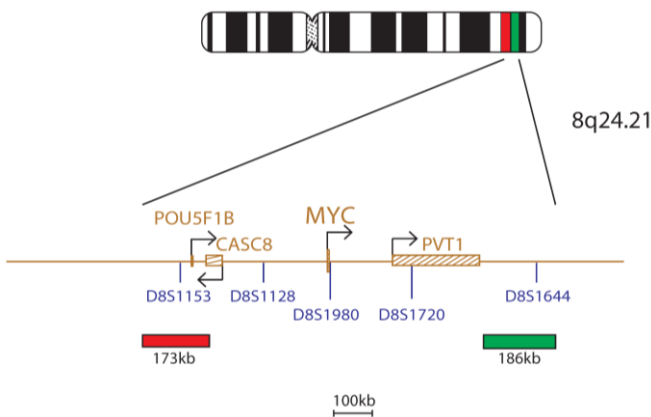
Intended Use

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

Probe Specification

cMYC (MYC) Breakpart

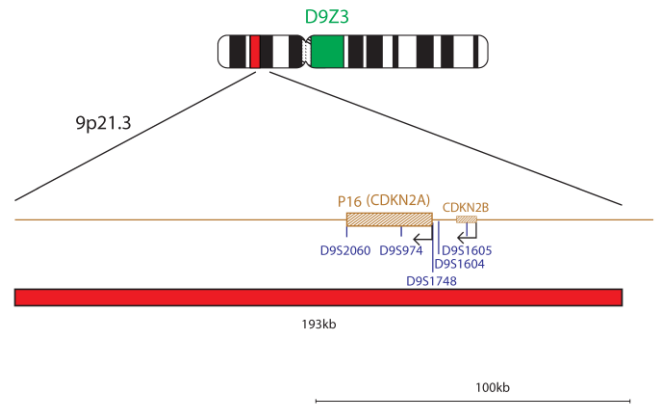
cMYC, 8q24.21, Red
cMYC, 8q24.21, Green



The cMYC product consists of a 173kb probe labelled in red, located centromeric to MYC gene that includes the marker D8S1153 and a green probe covering a 186kb region located telomeric to MYC gene, including the marker D8S1644.

P16 (CDKN2A) Deletion

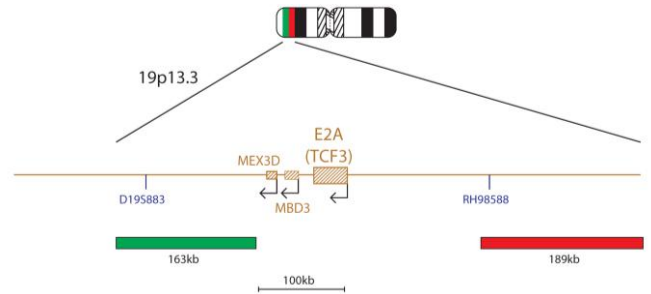
P16, 9p21.3, Red
D9Z3, 9q12, Green



The P16 (CDKN2A) product consists of a 193kb probe labelled in red that covers the P16 (CDKN2A) gene and the flanking regions. The mix also contains a control probe for chromosome 9 (D9Z3, the heterochromatic block at 9q12) labelled in green.

E2A (TCF3) Breakpart

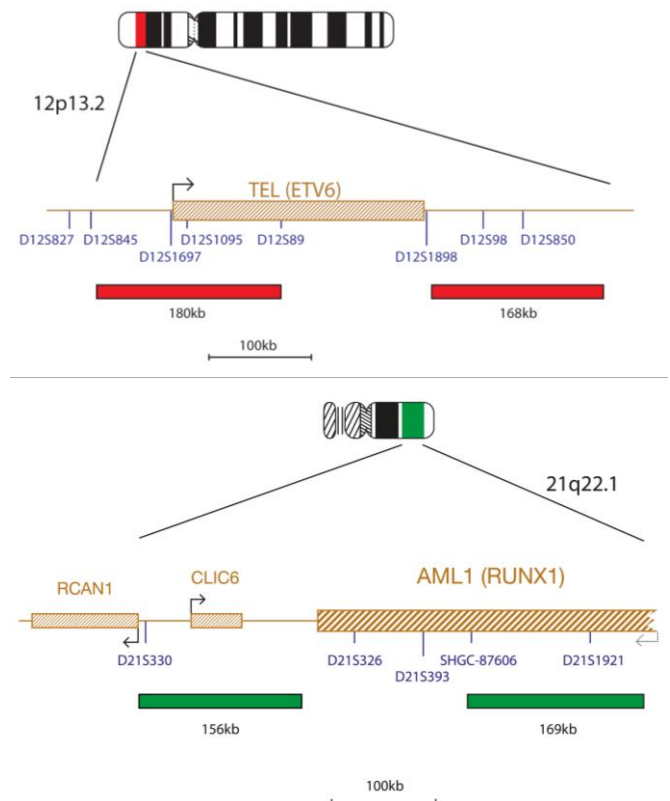
E2A, 19p13.3, Red
E2A, 19p13.3, Green



The E2A (TCF3) product consists of a 189kb probe, labelled in red, located centromeric to the E2A (TCF3) gene, including the RH98588 marker, and a green probe covering a 163kb region located telomeric to the E2A gene, including the D19S883 marker.

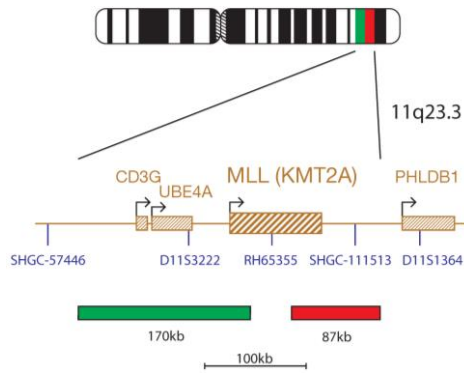
TEL/AML1 Translocation, Dual Fusion

TEL, 12p13.2, Red
AML1, 21q22.1, Green



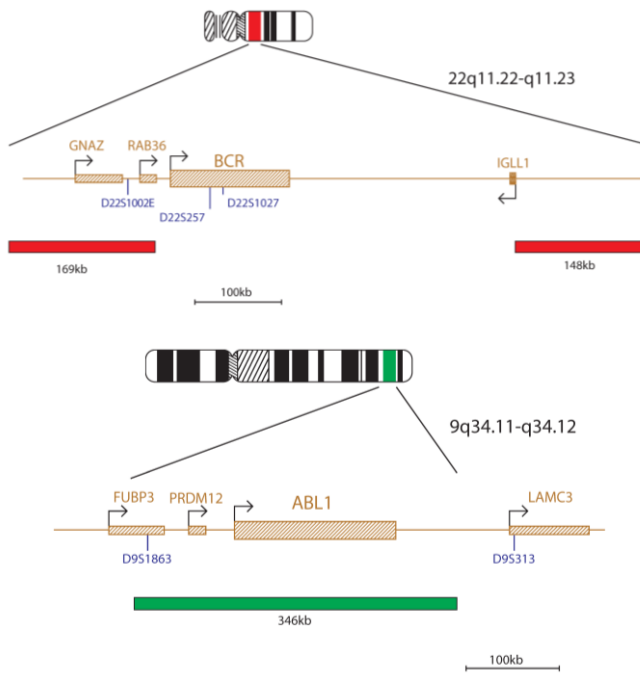
The TEL probe mix, labelled in red, contains a probe covering a 180kb region that includes the telomeric region of the TEL (ETV6) gene and another probe that extends 168kb on the centromeric side of the gene. For AML1, there are two probes, labelled in green, one covering a 156kb region centromeric to the AML1 (RUNX1) gene, including the CLIC6 gene and a second probe covering a 169kb region, including the marker D21S1921.

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



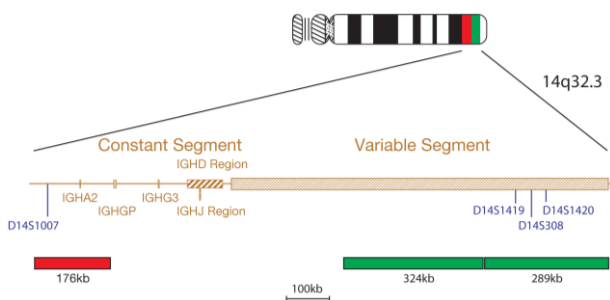
The MLL product consists of an 87kb probe, labelled in red, covering a region telomeric to the MLL (KMT2A) gene including the marker SHGC-111513 and a green probe covering a 170kb region centromeric to the MLL gene spanning the CD3G and UBE4A genes.

BCR/ABL Translocation, Dual Fusion
 BCR, 22q11.22-q11.23, Red
 ABL1, 9q34.11-q34.12, Green



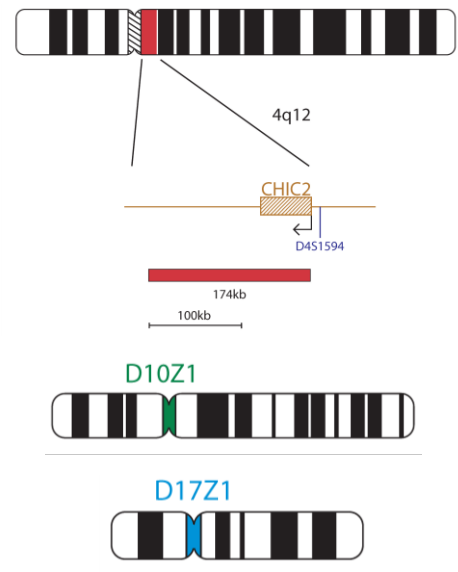
Two probes are designed around the BCR gene. The first probe extends 169Kb centromeric to the BCR gene and contains the genes GNAZ and RAB36 and the second probe covers a 148Kb region, which is positioned telomeric to the BCR gene. Both are labelled in red and are orientated such that breakpoints in either mBCR or MBCR will result in a fusion signal. A 346kb green probe covers the ABL1 gene and the flanking regions.

IGH Breakapart
 IGHC, 14q32.3, Red
 IGHV, 14q32.3, Green



The IGH product consists of a 176kb probe, labelled in red, covering part of the Constant region of the gene and a green probe, covering part of the Variable segment of the gene.

Hyperdiploidy
 CHIC2, 4q12, Red
 D10Z1, 10p11.1-q11.1, Green
 D17Z1, 17p11.1-q11.1, Blue



The ALL panel has been designed so that hyperdiploidy can be determined for chromosomes 4, 10 and 17 using CHIC2 probe for chromosome 4 along with centromeric probes for chromosomes 10 and 17. In addition, probes for IGH and AML1 will enumerate chromosomes 14 and 21 (squares 8 and 5 respectively), whilst cMYC on square 1 will enumerate chromosome 8.

Materials Provided

- Each kit contains the following reagents, which are sufficient for either 2 (RU-PMP 030), 5 (RU-PMP 031) or 10 (RU-PMP 032) devices:
- 2, 5 or 10 Multiprobe ALL Panel v2 (RUO) devices coated with directly labelled probes.
 - 4, 7 or 12 glass slides printed with a special 8-square template.
 - 500µl Hybridisation Solution (Formamide, Dextran Sulphate, SSC).
 - 1 Cytocell Slide Surface Thermometer.
 - 1 Cytocell Hybridisation Chamber.

*20 device kits (RU-PMP 033) is supplied as 4x5 Multiprobe ALL Panel v2 (RUO) devices.

Warnings and Precautions

- For research use only. Not for use in diagnostic procedures. For laboratory professional use only.
- Handle Multiprobe ALL Panel v2 (RUO) devices with care; wear gloves and a lab coat.
- 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.
- DAPI is a potential carcinogen. Handle with care; wear gloves and a lab coat.
- Follow local disposal regulations for your location along with recommendations in the Safety Data Sheet to determine the safe disposal of this product.
- Operators must be capable of visually distinguishing between red, blue and green.
- Room temperature (RT) = 15°C to 25°C.

Storage and Handling

The Multiprobe ALL Panel v2 (RUO) kit should be stored at 2-8°C until the expiry date indicated on the kit label. **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.

Protocol Recommendations

Equipment and Materials Necessary but not Supplied

- 500µl DAPI Antifade ES counterstain (0.125µg/ml DAPI (4,6-diamidino-2-phenylindole) in glycerol-based mounting medium).
- Hotplate (with a solid plate and accurate temperature control up to 80°C).
- Variable volume micropipettes and tips range 1µl - 200µl.
- Water bath with accurate temperature control at 72°C.
- Microcentrifuge tubes (0.5ml).
- Fluorescence microscope (Please see Fluorescence Microscope Recommendation section).
- Plastic or glass coplin jars.
- Forceps.
- Fluorescence grade microscope lens immersion oil.
- Bench top centrifuge.
- Microscope slides.
- Timer.
- 37°C 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. The aqua fluorophore has specificity to the Aqua and DEAC spectrum (single bandpass Aqua or DEAC filter is required).

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 ALL Panel v2 (RUO) 8-square template slides according to Cytocell's protocol below. Baking or otherwise ageing slides is not recommended as it may reduce signal fluorescence.

Multiprobe ALL Panel v2 (RUO) Protocol

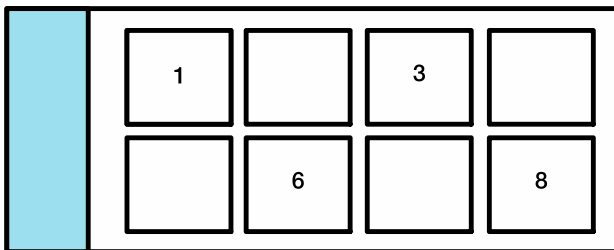
Please note: The probes used on the Multiprobe ALL Panel v2 (RUO) device are directly labelled with fluorophores, which are light sensitive. Ensure that exposure of the probes to laboratory lights is limited at all times (it is not necessary to work in the dark).

1. Slide preparation

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

- 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.
- Spotting of 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.



- 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 Multiprobe ALL Panel v2 (RUO) device and template slide

- Ensure that the Hybridisation Chamber is in the 37°C water bath and allow it to equilibrate to 37°C (+/- 1°C). This may take up to an hour if the water bath has been switched on from cold.
- Mix the hybridisation solution by repeated pipetting and pre-warm a 25µl aliquot per device to 37°C. Also pre-warm each device by placing it on a 37°C hotplate, label side down. Do not touch the raised surfaces of the device.
- Immerse template slides containing fixed samples in 2xSSC for 2 minutes at room temperature (RT) without agitation.
- Whilst the device is still at 37°C, 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 37°C hotplate to warm up.
- 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 37°C hotplate.

3. Positioning of template slide over the device

- 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).

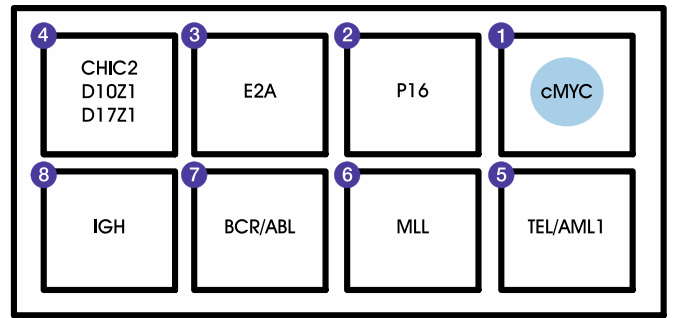


Figure 1. Location of the probes on the Multiprobe ALL Panel v2 (RUO). To help locate square 1, its position on the device has been marked with a coloured label.

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

4. Instructions for use of the Cytocell Slide Surface Thermometer

- The temperature of the 75°C hotplate should be checked with the Cytocell Slide Surface Thermometer before proceeding to denaturation.
 - 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:**
- 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.
 - 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.

- 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.
- Denature on the hotplate at 75°C (+/- 1°C) 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 37°C (+/- 1°C) water bath (non-stirring) overnight.

Please note:

- Do not seal the lid on the hybridisation chamber.
- Do not place a lid on the water bath.
- Do not hybridise in an incubator.
- 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.

- Remove the device carefully from the slide.
- Immerse the slide in 0.4xSSC (pH 7.0) at 72°C (+/- 1°C) for 2 minutes without agitation.
- 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

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

cMYC Breakpart

In a normal cell two red/green (or fused yellow) signals are expected (2Y). In a cell with cMYC 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 8 (1R, 1G, 1Y).

P16 Deletion

In a normal cell there should be two red and two green signals (2R, 2G). A cell with a hemizygous deletion of the P16 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).

E2A Breakpart

In a normal cell two red/green (or fused yellow) signals are expected (2Y). In a cell with E2A 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 19 (1R, 1G, 1Y).

Hyperdiploidy

In a normal cell two red, two green and two blue signals are expected (2R, 2G, 2B). In a cell with more than two copies of these chromosomes there should be multiple signals from the corresponding chromosomes.

TEL/AML1 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(12;21)(p13.2;q22.12) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosomes 12 and 21 respectively (1R, 1G, 2Y).

MLL Breakpart

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).

BCR/ABL 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(9;22)(q34;q11) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosomes 22 and 9 respectively (1R, 1G, 2Y).

IGH Breakpart









In a normal cell two red/green (or fused yellow) signals are expected (2Y). In a cell with monoallelic IGH 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 14 (1R, 1G, 1Y). In the event of a biallelic translocation no fused signals would be present, two distinct red and two green signals should be observed (2R, 2G).

Additional Information

For additional product information please contact the Cytocell Technical Support Department.

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Guide to Symbols

REF	en: Catalogue number
	en: Batch code
	en: Consult instructions for use
	en: Manufacturer
	en: Use-by date
	en: Temperature limit
	en: Keep away from sunlight
	en: Contains sufficient for <n> tests
	en: Contents

Patents and Trademarks

Cytocell is a registered trademark of Cytocell Ltd.



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