A Sysmex Group Company

CytoCell

REF: RU-PMP 016 / RU-PMP 017 / RU-PMP 018 / RU-PMP 020

Multiprobe CLL Panel (RUO)

Research Use Only

ENGLISH

Further information available at www.ogt.com

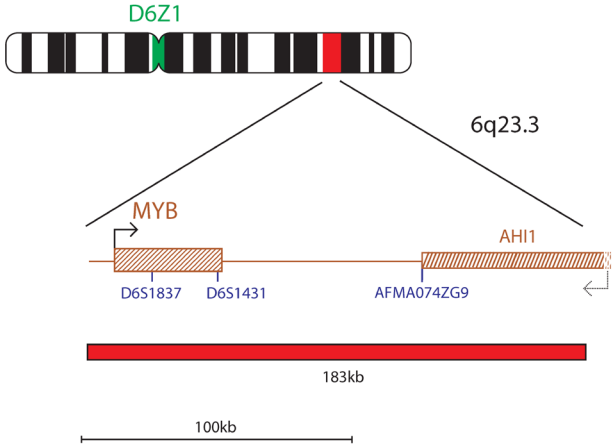
To maintain optimum storage conditions, when not in use ensure the kit is stored in the additional zip-lock bag provided. Ensure the silica gel remains in the kit box at all times and is not discarded.

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

MYB Deletion
MYB, 6q23.3, Red
D6Z1, 6p11.1-q11.1, Green

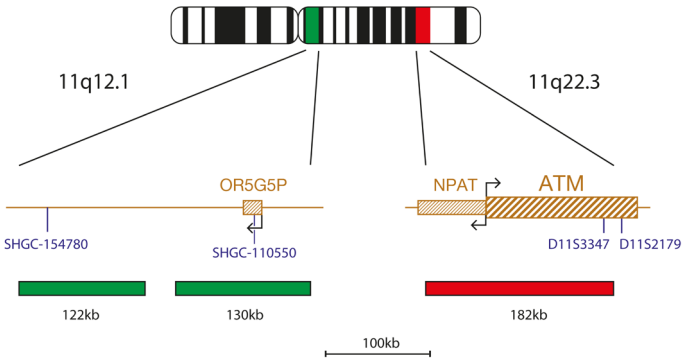


The MYB probe is 183kb in length and is labelled in red. It covers the MYB gene and continues into the centromeric region of the AHI1 gene beyond marker AFMA074ZG9. The probe mix also contains a control probe for the 6 centromere (D6Z1) labelled in green.

Chromosome 12 Alpha Satellite D12Z3, 12p11.1-q11.1, Red

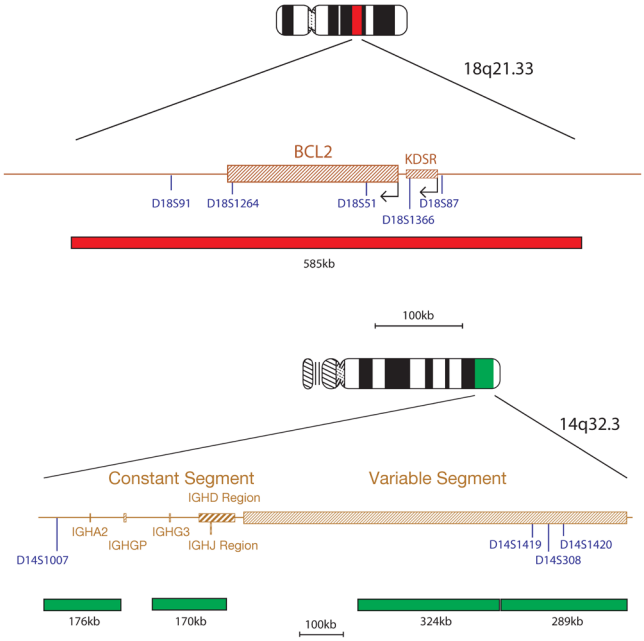
The Chromosome 12 Alpha Satellite Probe is a repeat sequence probe, labelled in red, which recognises the centromeric repeat sequence D12Z3.

ATM Deletion
ATM, 11q22.3, Red
11q12.1, Green



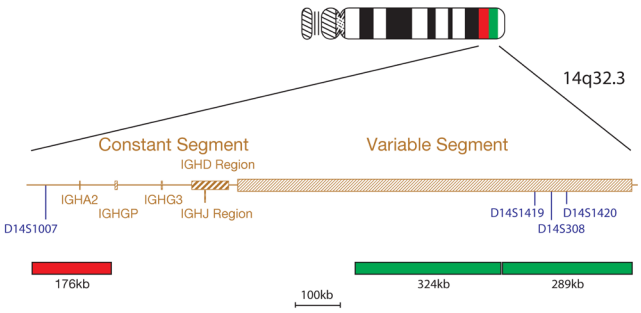
The ATM probe is 182kb in length and is labelled in red. It covers the telomeric end of the NPAT gene and the centromeric end of the ATM gene to beyond the D11S3347 marker. The probe mix also contains a 11q12.1 control probe labelled in green, covering a 122kb region including the SHGC-154780 marker and a 130kb region including the SHGC-110550 marker.

IGH/BCL2 Translocation, Dual Fusion
BCL2, 18q21.3 Red
IGH, 14q32.3 Green



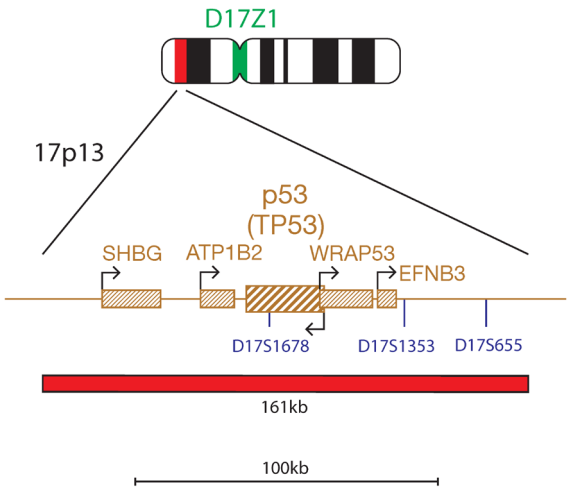
The IGH/BCL2 product consists of probes, labelled in green, covering the Constant, J, D and Variable segments of the IGH gene, and a 585kb probe, labelled in red, covering the BCL2 and KDSR genes.

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



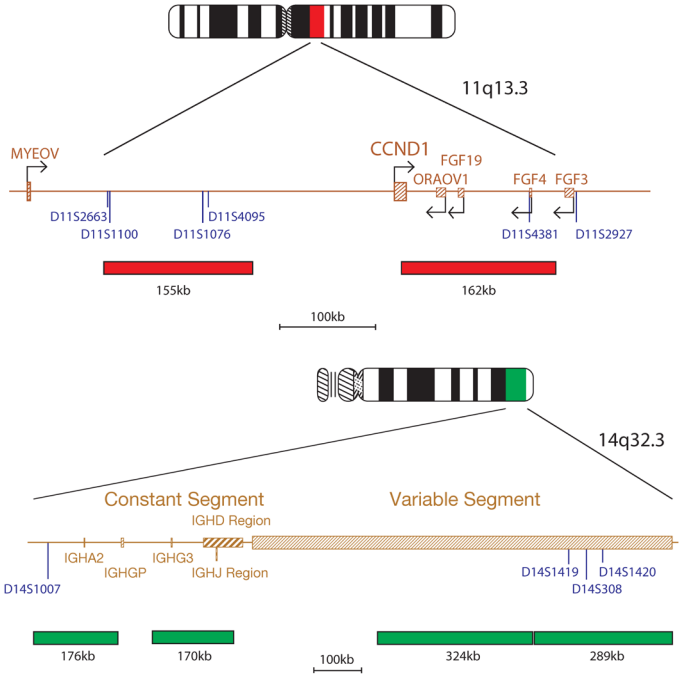
The IGH product consists of a 176kb probe, labelled in red, covering part of the Constant region of the gene and a green probe, covering part of the Variable segment of the gene.

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



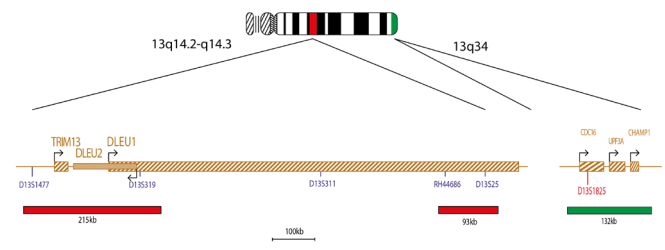
The P53 probe is 161kb in length and is labelled in red. It covers the whole P53 (TP53) gene and flanking regions. The probe mix also contains a control probe for the 17 centromere (D17Z1) labelled in green.

IGH/CCND1 Translocation, Dual Fusion
CCND1, 11q13.3, Red
IGH, 14q32.3, Green



The IGH/CCND1 product consists of probes, labelled in green, covering the Constant, J, D and Variable segments of the IGH gene, and CCND1 probes, labelled in red. The CCND1 probe mix contains a 155kb probe centromeric to CCND1 gene, covering a region between the D11S2663 and the D11S4095 markers, and a second probe (162kb) covering the telomeric end of CCND1 gene and the region up to FGF3 gene.

13q14.3 Deletion
13q14.2-q14.3, Red
13qter, 13q34, Green



The 13q14.3 probe, labelled in red, covers the D13S319 and D13S25 markers. The 13qter subtelomere specific probe (clone 163C9), labelled in green, allows identification of chromosome 13 and acts as a control probe.

Materials Provided
Each kit contains the following reagents, which are sufficient for either 2 (RU-PMP 018), 5 (RU-PMP 017) or 10 (RU-PMP 016) devices*:
1. 2, 5 or 10 Multiprobe CLL Panel (RUO) devices coated with directly labelled probes
2. 4, 7 or 12 glass slides printed with a special 8-square template
3. 500µl Hybridisation Solution B (Formamide, Dextran Sulphate, SSC)
4. 1 CytoCell Slide Surface Thermometer
5. 1 CytoCell Hybridisation Chamber

*20 device kit (RU-PMP 020) is supplied as 4x5 Multiprobe CLL Panel (RUO) devices.

Warnings and Precautions
1. For research use only. Not for use in diagnostic procedures.
2. For laboratory professional use only.
3. Handle Multiprobe CLL Panel (RUO) devices with care; wear gloves and a lab coat.
4. Hybridisation Solution B contains formamide, which is a teratogen; do not breathe fumes or allow skin contact. Handle with care; wear gloves and a lab coat.
5. DAPI is a potential carcinogen. Handle with care; wear gloves and a lab coat.
6. Follow local disposal regulations for your location, along with recommendations in any applicable Safety Data Sheets, to determine the safe disposal of this product and the safe disposal of any accessories, consumables, and related waste substances.
7. Operators must be capable of visually distinguishing between red, blue and green.
8. Room temperature (RT) = 15ºC to 25ºC.

Storage and Handling
The Multiprobe CLL 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 6 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. Fluorescence grade glass coverslips (24 x 50 mm)
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 8-square template slides according to CytoCell protocol below. Baking or otherwise ageing slides is not recommended as it may reduce signal fluorescence.

Multiprobe CLL Panel (RUO) Protocol

Please note: The probes used on the Multiprobe CLL 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).

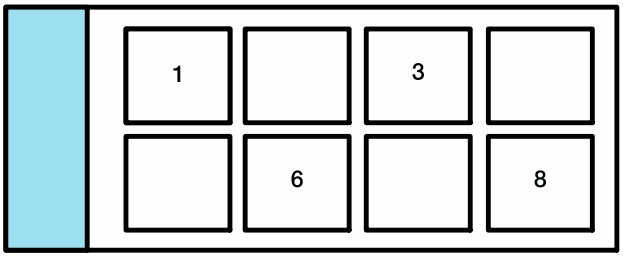
1. Slide preparation
a) Clean a template slide. Soak the template slide for 2 minutes in 100% methanol and polish dry with a clean soft tissue.
b) Establish the correct mitotic index. It is important that the intended sample has a sufficiently high mitotic index to allow analysis of the sample. To check 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 CLL Panel (RUO) device and template slide

a) Ensure that the Hybridisation Chamber is in the 37ºC water bath and allow 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).

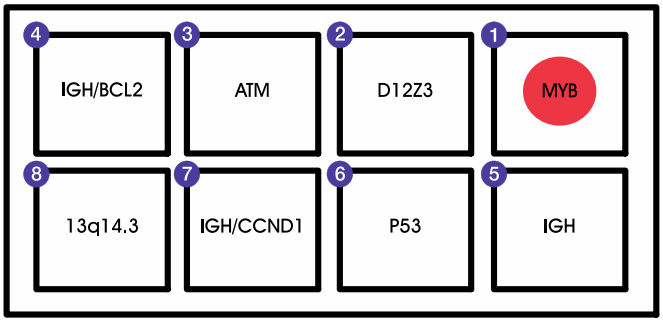


Figure 1. Location of the probes on the Multiprobe CLL Panel (RUO). To help locate square 1, its position on the device has been marked with a coloured label.

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), 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

MYB Deletion

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

Chromosome 12 Enumeration

In a normal cell two red signals should be observed (2R). In a cell with more than two copies of chromosome 12 there should be multiple red signals.

ATM Deletion

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

IGH/BCL2 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(14;18)(q32.3;q21) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosomes 18 and 14 respectively (1R, 1G, 2Y).

IGH Breakapart
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).

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).

IGH/CCND1 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(11;14)(q13;q32.3) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosomes 11 and 14 respectively (1R, 1G, 2Y).

13q14.3 Deletion
In a normal cell there should be two red and two green signals (2R, 2G). A cell with a hemizygous deletion of the 13q14.3 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). 13q deletions in CLL are recognised as being heterogenous; small deletion within the 13q region may result in a small residual signal with this probeset.

Additional Information
For additional product information please contact the CytoCell Technical Support Department.
T: +44 (0)1223 294048
E: techsupport@cytocell.com
W: www.ogt.com

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.

Cytocell Ltd
Oxford Gene Technology,
418 Cambridge Science Park,
Milton Road,
Cambridge, CB4 0PZ, UK
T: +44(0)1223 294048
F: +44(0)1223 294986
E: probes@cytocell.com
W: www.ogt.com