IGH-BCL2 Fusion/Translocation FISH Probe
Ref: MAD-050M (10 test)

INTRODUCTION:

Members of the BCL2 family of proteins play important roles in the control of programmed cell death (apoptosis). They comprise proteins that facilitate apoptosis, as well as apoptosis inhibitors.

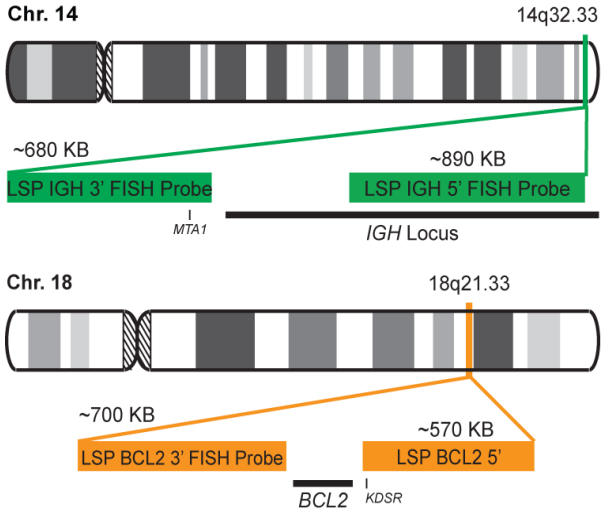
Lymphomas are malignant conditions of the lymphatic part of the circulatory system.

One of the most frequent subtypes of lymphoma in adults is follicular lymphoma, a cancer originating in certain B cells that is often symptomless in its early stages. Nearly half of lymphomas diagnosed in adults are of the follicular subtype. One of the widely recognized molecular characteristics of this subtype of the condition is that BCL2 is regularly dysregulated in follicular lymphomas, most often by gene translocation. In fact, *BCL2* protein has been detected in virtually all transformed cells in samples from follicular lymphoma patients, whereas it was not seen in non-neoplastic cells or in normal lymph nodes. One particularly common rearrangement is a reciprocal translocation, in which the *BCL2* gene, normally on chromosome 18, is relocated to the *immunoglobulin heavy chain* (*IGH*) gene region on chromosome 14 and is overexpressed under the influence of an enhancer element on that locus. However, fusion of *BCL2* with other genes, including *AFF3* and the *IGHL@* and *IGK@* gene loci, has also been observed.

INTENDED USE:

The IGH-BCL2 Fusion/Translocation FISH probe kit is designed to detect rearrangements involving regions of the human IGH locus, located on chromosome band 14q32.33, and of the human *BCL2* gene on 18q21.33. The kit contains two differentially labeled Locus Specific Probe (LSP) pairs. One set of probes covers the 5' (start) portion of the *BCL2* gene and some upstream untranslated genomic sequence as well as sequences downstream of the 3' (end) part of the gene. The two probes are flanking sequences on both sides and inside the *BCL2* gene where breakpoint clusters and individual breakpoints have been found.

The second probe pair spans the *immunoglobulin heavy chain* gene locus (*IGH@*) and consists of a portion matching the 5' (start) region encoding variable gene segments (*IGHV*), and a part that covers the 3' (end) and downstream sequences. This probe pair is flanking an area of the *IGH* gene comprising constant gene segments (*IGHC*) as well as the delta segment (*IGHD*), joining region (*IGHJ*) and epsilon domain (*IGHE*). Almost all known breakpoints in *IGH@* have been mapped to this region.



PROBE DESCRIPTION:

The BCL2 Probe of the IGH-BCL2 Fusion/Translocation FISH Probe Kit labeled with the fluorochrome CytoOrange covers some genomic sequences adjacent to the 5' (start) portion of the BCL2 gene; it also covers sequences downstream of the 3' end of the gene. The IGH Probe of the IGH-BCL2 Fusion/Translocation FISH Probe Kit labeled with the fluorochrome CytoGreen covers the 5' and the center sequences of the IGH locus, and it also covers the 3' (end) part and the neighboring downstream region.

	Fluorochrome	Excitation	Emission
IGH Probe	CytoGreen	495 nm	518 nm
BCL2 Probe	CytoOrange	551 nm	575 nm

REFERENCE AND PRESENTATION:

MAD-050M – **IGH-BCL2 Fusion/Translocation FISH Probe Kit** - 10 tests in ready-to-use format.

RECOMMENDED WORKING PROTOCOL:

White cells fixation:

- Isolate the white cells population by Buffy Coat: start from 1-3 ml of blood / bone marrow with EDTA and centrifuge at 1500-2000 xg for 10-15 min at room temperature. This process will separate an upper plasma phase, a lower one with the red cells population and a thin interface between both containing the white cells (buffy coat). (In a typical clinical centrifuge 1500-2000 xg equals 3000-3400

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rpm).

- Collect the intermediate white population with a Pasteur pipette and transfer to a new 15 ml centrifuge tube.

- Add 3-5 ml of 7.5 mM KCl hypotonic solution (2.8 g of KCl in 500 ml of distilled water). Drop the solution smoothly through the walls of the tube. Mix gently by several inversions.

- Incubate 20 min at 37 ° C to lyse the erythrocytes (red cells population).

- Add a few drops of freshly prepared Methanol: Acetic (3: 1) shake gently and centrifuge at 2000 rpm for 5 min.

- Remove the supernatant and leave a residual volume of liquid together with the cells pellet.

- Suspend the cells pellet in the remaining liquid until getting a homogeneous suspension.

- Fix the cells by adding 3-5 ml of Methanol: Acetic (3: 1) to the cell suspension, dropping dropwise through the walls of the tube and shaking gently to avoid lumping. Centrifuge and remove the supernatant.

- Repeat the previous step 2-3 times until the cell button is white color (the red cells have been removed).

- Finally add 1 ml of Methanol: Acetic (3: 1) to the cells pellet, suspend it by mixing gently to get a homogeneous suspension and transfer to a 2ml tube. This cells suspension can be stored at -20ºC for several days/weeks.

Hybridization protocol:

Before the hybridization step perform the following procedure:

- Apply a drop of the cell suspension (preserved in Methanol / Acetic) on the surface of an slide.

- Let it air-dry.

- Place the slide on a hotplate at 90 °C for 5 minutes.

- Wash the slide through ethanol 90° and absolute ethanol for a few seconds.

- Let it air dry and continue with the hybridization protocol following the instructions recommended by the manufacturer.

Note: *It is recommended to mix the FSIH probe before each use by pipetting several times.*

- Add 10 µl of the probe to the slide containing the fixed cells, cover with a coverslip and seal hermetically with fixogum.

- Put the slide in a hybridizer or hotplate and perform the hybridization protocol as follows:

Denaturation: 80°C, 5 min
Hybridization: 43°C, 16 hours

Perform the following procedure:

- Once the hybridization is finished remove the coverslip and wash the slide with Wash Buffer for 3 minutes at RT.

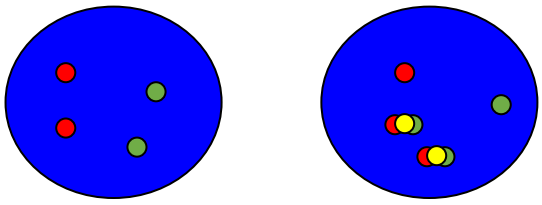
- Wash for 2 minutes at 75 ° C in Wash Buffer.

- Wash for 1 minute at RT in distilled water.

- Let it air dry in the darkness.

- Mounting with DAPI anti-FADE mounting medium.

INTERPRETATION:



Normal Pattern | Abnormal Pattern

Note: Others type of abnormal patterns could be found.

For the interpretation of the results of the fluorescence hybridization, it is recommended to apply the interpretation criteria in force and accepted worldwide.

WARNINGS AND PRECAUTIONS:
1. Avoid the contact of the reagents with the eyes and the mucous membranes. If the reagents come into contact with sensitive areas, wash them with plenty of water.
2. This product is harmful if swallowed.
3. Check with the local or state authorities about the recommended disposal method.
4. Avoid the microbial contamination of the reagents.

SAFETY RECOMMENDATIONS: The product is only intended for professional laboratory purposes, and it is not intended for pharmacological, home or any other type of use. The current version of the material safety data sheet of this product can be downloaded by searching its reference at www.vitro.bio or can be requested at regulatory@vitro.bio.

STORAGE: Keep refrigerated between 2 and 8ºC until the expiration date of the product. **DO NOT FREEZE THE PRODUCT.**

BIBLIOGRAPHY

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2) Willis TG & Dyer MJ. Blood. 96(3):808-22 (2000).
3) Ngan BY, et al. N Engl J Med. 318(25):1638-44 (1988).
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5) Vaandrager JW, et al. Genes Chromosomes Cancer. 27(1):85-94 (2000).

LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

	Expiration date
	Temperature limit
	Manufacturer
	Sufficient content for <n> assays
	Catalog number
	Lot code
	Refer to the instructions of use
	Medical product for *in vitro* diagnosis.
	Material safety data sheet
	Keep away from sunlight

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