IGH Break Apart FISH Probe
Ref: MAD-049M (10 test)

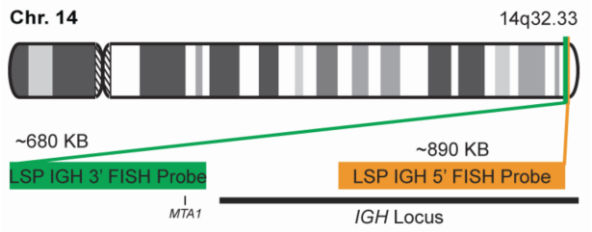
INTRODUCTION:

Rearrangements involving the IGH locus (or immunoglobulin heavy locus) have been observed in many types of leukemias and lymphomas. It represents the prototype for translocations in B-cell non-Hodgkin lymphomas, in which it has been detected in up to 50% of the cases, whereas reciprocal and specific translocations with different chromosomal loci have been characterized and considered as pathognomonic for different histological subtypes.

INTENDED USE:

The IGH Break Apart FISH probe is designed to detect rearrangements in the human IGH locus located in the chromosome band 14q32.33.

Aside from detecting breaks that can lead to translocation of parts of the locus, to its inversion or to its fusion with other genes, the probe can also be used to identify other aberrations of IGH such as deletions or amplifications.



PROBE DESCRIPTION:

The telomeric 5' Fragment of the IGH Break Apart FISH probe labeled with the fluorochrome CytoOrange covers the 5' portion and the center of the locus of the IGH gene, whereas the centromeric 3' fragment of the IGH Break Apart FISH probe labeled with CytorGreen covers some distal sequences (3' end) of the gene.

Both loci are flanking sequences of the BCL2 gene, in which numerous cut-off points have been observed.

	Fluorochrome	Excitation	Emission
3' Fragment	CytoGreen	495 nm	518 nm
5' Fragment	CytoOrange	551 nm	575 nm

REFERENCE AND PRESENTATION:

MAD-049M – **IGH Break Apart 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 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.

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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.

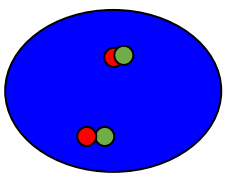
- Wash for 2 minutes at 75 ° C in Wash Buffer.

- Wash for 1 minute at RT in distilled water.

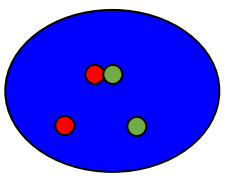
- Let it air dry in the darkness.

- Mounting with DAPI anti-FADE mounting medium.

INTERPRETATION:



Cell without IGH translocation



Cell with IGH translocation

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

1: Ventura RA, Martin-Subero JI, Jones M, McParland J, Gesk S, Mason DY, Siebert R. FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. J Mol Diagn. 2006 May;8(2):141-51.
2: Belaud-Rotureau MA, Parrens M, Dubus P, Turmo M, Lacroute G, Taine L, Vago P, De Mascarel A, Merlio JP. [Interphase FISH analysis on histologic sections of fixed tissues for t(11;14) (q13;q32) detection in mantle cell lymphoma and t(8;14)(q24;q32) in Burkitt's lymphoma]. Ann Pathol. 2002 Apr;22(2):145-9.
3. Kocjan G. Best Practice No 185. Cytological and molecular diagnosis of lymphoma. J Clin Pathol. 2005 Jun;58(6):561-7
4. Remstein ED, Kurtin PJ, Buño I, Bailey RJ, Proffitt J, Wyatt WA, Hanson CA, Dewald GW. Diagnostic utility of fluorescence in situ hybridization in mantle-cell lymphoma. Br J Haematol. 2000 Sep;110(4):856-62

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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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