DLEU1/ZBTB16 FISH Probe
Ref: MAD-048M (10 test)

INTRODUCTION:

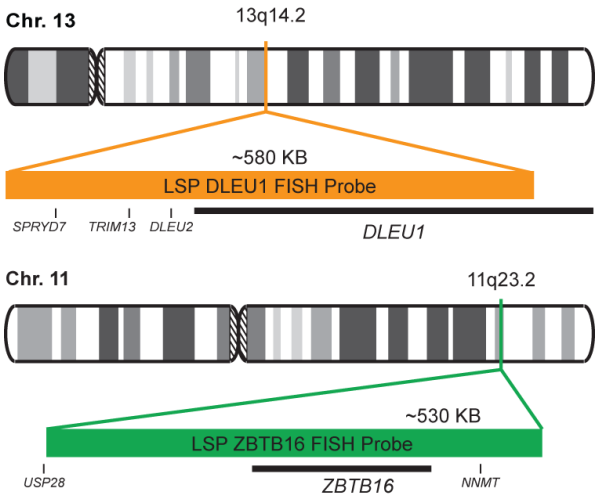
The DLEU1 gene was originally identified as a potential tumor suppressor gene.

The ZBTB16 gene is a member of the Kruppel C2H2 type zinc finger protein family and encodes a transcription factor containing nine Kruppel-type zinc finger domains at the C-terminus. The ZBTB16 protein is located in the cell nucleus and is involved in the progression of the cell cycle, interacting with histone deacetylases.

Rearrangements and abnormal expression of the DLEU1 gene region – also known as BCMS, DLB1, LEU1, LEU2, XTP6, BCMS1, DLEU2, LIN C00021 or NCRNA00021 – have been observed in B-cell chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and other malignancies. Fusions and aberrant expression of the ZBTB16 gene – also known as PLZF or ZNF145 – have been reported in acute promyelocytic leukemia and other neoplasias.

INTENDED USE:

The DLEU1/ZBTB16 FISH Probe Kit is designed to detect the human *DLEU1* gene, located on chromosome band 13q14.2, and the *ZBTB16* gene on chromosome band 11q23.2.



PROBE DESCRIPTION:

The DLEU1 Probe of the DLEU1/ZBTB16 FISH Probe kit labeled with the fluorochrome CytoOrange covers a chromosomal region which includes the majority of the DLEU1 gene and genomic sequences adjacent to the 5' end of the gene. The ZBTB16 Probe of the DLEU1/ZBTB16 FISH Probe Kit labeled with CytoGreen e covers a chromosomal region which includes the entire ZBTB16 gene. Both probes are designed to measure the copy number of the human DLEU1 and ZBTB16 genes located on chromosome bands 13q14.2 and 11q23.2, respectively.

	Fluorochrome	Excitation	Emission
ZBTB16 Probe	CytoGreen	495 nm	518 nm
DLEU1 Probe	CytoOrange	551 nm	575 nm

REFERENCE AND PRESENTATION:

MAD-048M – **DLEU1/ZBTB16 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.

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- 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.

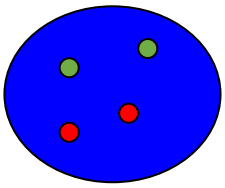
- Wash for 2 minutes at 75 ° C in Wash Buffer.

- Wash for 1 minute at RT in distilled water.

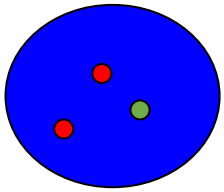
- Let it air dry in the darkness.

- Mounting with DAPI anti-FADE mounting medium.

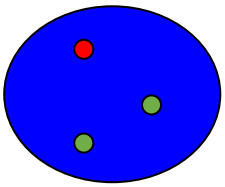
INTERPRETATION:



Normal Pattern



Abnormal Pattern



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) Stevens-Kroef M, et al. Cancer Genet Cytogenet. 195(2):97-104 (2009).
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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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