IGH-CCND1 Fusion/Translocation FISH Probe
Ref: MAD-051M (10 test)

INTRODUCTION:

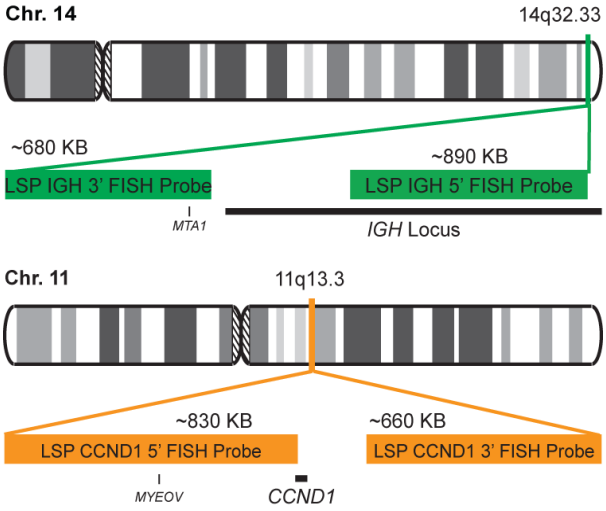
Cyclin D1, also called B-cell leukemia/lymphoma 1 (BCL1) or parathyroid adenomatosis 1 (PRAD1), belongs to a family of highly conserved proteins that serve as key regulators of the cell cycle. They form complexes with, and thereby regulate the activity of cyclin-dependent kinases (CDKs), a family of protein kinase enzymes required for progression through the cell cycle.

Cyclin D1 is overexpressed in many tumor types. For example, it has been found amplified, and proven to be a marker of poor prognosis, in a percentage of breast cancer cases. CCND1 is also aberrantly activated in a number of hematological malignancies. It is mainly found in mantle cell lymphoma (MCL), a relatively rare non-Hodgkin's lymphoma type. The most common translocation in MCL is one in which *CCND1* is moved to the *IGH* locus on chromosome 14; juxtaposition of the two genes leads to activation of the *CCND1* gene via enhancer elements in the *IGH* locus. However, fusion of *CCND1* with other genes, including *FSTL3* and the *IGHL@* and *IGK@* gene loci, has also been observed. CCND1 activation by translocation also occurs in B-prolymphocytic leukemia, plasma cell leukemia, splenic lymphoma with villous lymphocytes and sometimes in chronic lymphocytic leukemia and multiple myeloma.

Detection of *CCND1* gene translocation is now widely recommended for the discernment of specific lymphoma subtypes. Furthermore, it is a predictor of improved survival in multiple myeloma patients.

INTENDED USE:

The IGH-CCND1 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 *CCND1* gene on 11q13.3. The kit contains two differentially labeled Locus Specific Probe (LSP) pairs. One set of probes covers the 5' (start) portion of the *CCND1* 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 in the *CCND1*gene in which both a 3' breakpoint cluster as well as several individual breakpoints have been observed.



PROBE DESCRIPTION:

The IGH Probe of the IGH-CCND1 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. The CCND1 Probe of the IGH-CCND1 Fusion/Translocation FISH Probe Kit labeled with the fluorochrome CytoOrange covers some genomic sequences adjacent to the 5' (start) portion of the CCND1 gene; it also covers sequences downstream of the 3' end of the gene. The probe set is optimized to reveal translocations between the two regions.

	Fluorochrome	Excitation	Emission
IGH Probe	CytoGreen	495 nm	518 nm
CCND1 Probe	CytoOrange	551 nm	575 nm

REFERENCE AND PRESENTATION:

MAD-051M – **IGH-CCND1 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 rpm).

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- 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.

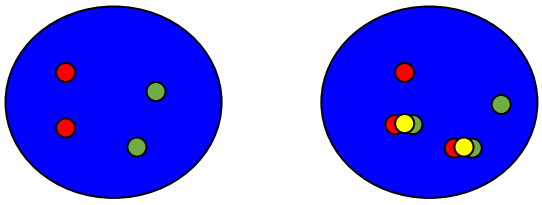
- Wash for 2 minutes at 75 ° C in Wash Buffer.

- 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

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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) Siegert JL, et al. Oncogene. 19(50):5703-11 (2000).
2) Motokura T, et al. Nature. 350(6318):512-5 (1991).
3) Rimokh R, et al. Blood. 83(12):3689-96 (1994).
4) Brizard F, et al. Leuk Lymphoma. 25(5-6):539-43 (1997).
5) Fonseca R, et al. Leukemia. 23(12):2210-21 (2009)

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