ZytoMation

BCL2 Dual Color Break Apart FISH Probe

REF Z-2306-5.1ML Σ Up to 20 (5.1 ml)

For the qualitative detection of translocations involving the human BCL2 gene at 18q21.33 by fluorescence *in situ* hybridization (FISH) on automated Bond systems

In vitro diagnostic medical device
according to EU directive 98/79/EC

1. Intended use

The Zyto*Mation* BCL2 Dual Color Break Apart FISH Probe (**PL260**) is intended to be used for the qualitative detection of translocations involving the human BCL2 gene at 18q21.33 in formalin-fixed, paraffin-embedded specimens by fluorescence *in situ* hybridization (FISH). The probe is intended to be used in combination with the Bond FISH Kit (DS9636) on the automated Bond-MAX or Bond III system by Leica Biosystems.

Interpretation of the results must be made within the context of the patient´s clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

2. Clinical relevance

The BCL2 (B-cell CLL/lymphoma 2, a.k.a. PPP1R50) gene encodes a mitochondrial membrane protein that regulates apoptosis and is expressed in B-cells. Translocations involving the BCL2 gene are commonly identified in B-cell lymphomas. In particular, the translocation t(14;18)(q32.3;q21.3) has been identified in about 80% of follicular lymphoma (FL), in 20% to 30% of diffuse large B-cell lymphoma (DLBCL), and rarely in B-cell chronic lymphocytic leukemia (B-CLL). In FL this translocation is considered to be a cytogenetic hallmark. As a result of this rearrangement, the BCL2 gene is juxtaposed to the IGH (immunoglobulin heavy chain) locus at 14q32.33 which leads to overexpression of the anti-apoptotic protein BCL2, and finally to progression to lymphoma. Alternative BCL2 translocations to immunoglobulin light chain genes as well as non-IG translocation events have been reported. In DLBCL, BCL2 gene overexpression has been implicated in conferring resistance to chemotherapy and has been associated with poor prognosis. Hence, detection of BCL2 translocations by fluorescence *in situ* hybridization (FISH) may be of diagnostic and prognostic relevance.

3. Test principle

The fluorescence *in situ* hybridization (FISH) technique allows for the detection and visualization of specific nucleic acid sequences in cell preparations. Fluorescently-labeled DNA fragments, so called FISH probes, and their complementary target DNA strands in the preparations are co-denatured and subsequently allowed to anneal during hybridization. Afterwards, unspecific and unbound probe fragments are removed by stringency washing steps. After counterstaining the DNA with DAPI, hybridized probe fragments are visualized using a fluorescence microscope equipped with excitation and emission filters specific for the fluorochromes with which the FISH probe fragments have been directly labeled.

4. Reagents provided

The Zyto*Mation* BCL2 Dual Color Break Apart FISH Probe is composed of:

- ZyGreen (excitation 503 nm/emission 528 nm) labeled polynucleotides (~6.0 ng/µl), which target sequences mapping in 18q21.33* (chr18:60,046,152-60,589,273) proximal to the BCL2 breakpoint region (see Fig. 1).
- ZyOrange (excitation 547 nm/emission 572 nm) labeled polynucleotides (~2.5 ng/µl), which target sequences mapping in 18q21.33-q22.1* (chr18:60,994,528-61,658,503) distal to the BCL2 breakpoint region (see Fig. 1).
- Formamide based hybridization buffer

*according to Human Genome Assembly GRCh37/hg19

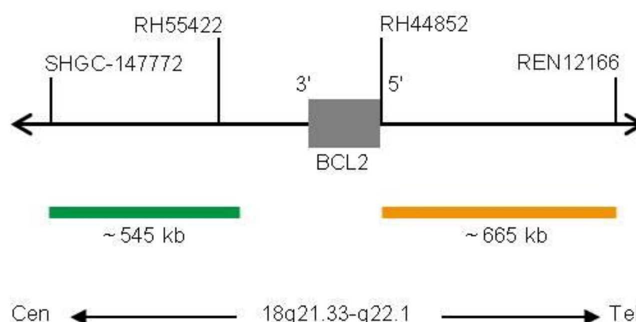

Fig. 1: BCL2 Probe map (not to scale)

The Zyto*Mation* BCL2 Dual Color Break Apart FISH Probe is available in one size:

- Z-2306-5.1ML: 5.1 ml (up to 20 reactions of 240 µl each)

5. Materials required but not provided

- A fully automated Bond-MAX system (Leica Biosystems)
- Bond FISH Kit (DS9636)
- DAPI/DuraTect-Solution (MT-0007-0.8)
- Positive and negative control specimens
- Microscope slides, positively charged
- Adjustable pipettes (25 µl, 1000 µl)
- Staining jars or baths
- Timer
- Ethanol or reagent alcohol
- Deionized or distilled water
- Coverslips (24 mm x 60 mm)
- Adequately maintained fluorescence microscope (400-1000x)
- Immersion oil approved for fluorescence microscopy
- Appropriate filter sets

For further information on materials required but not provided please refer to the instructions for use of the respective fully automated staining system.

6. Storage and handling

Store at 2-8°C in an upright position protected from light.
Use protected from light. Prior to opening the vial, shake down liquid. Return to storage conditions immediately after use. Do not use reagents beyond expiry date indicated on the label. The product is stable until expiry date indicated on the label when handled accordingly.

7. Warnings and precautions

- Read the instruction for use prior to use!
- Do not use the reagents after the expiry date has been reached!
- The probe should not be used in manual FISH procedures!
- This product contains substances (in low concentrations and volumes) that are harmful to health and potentially infectious. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
- If reagents come into contact with skin, rinse skin immediately with copious quantities of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents.
- Avoid cross-contamination of samples as this may lead to erroneous results.
- The probe should not be exposed to light, especially strong light, for a longer period of time, i.e., all steps should be accomplished, where possible, in the dark and/or using lightproof containers!

Hazard and precautionary statements:

The hazard determining component is Formamide.

Danger

H351	Suspected of causing cancer.
H360FD	May damage fertility. May damage the unborn child.
H373	May cause damage to organs through prolonged or repeated exposure.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.

8. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the FISH probes, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other specimens or fluids may produce artefacts or false results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the specimen.
- The probe should be used only for detecting loci described in 4. "Reagents provided".
- The performance was validated using the fully automated Bond-MAX system (Leica) and the procedures described in this instruction for use. Modifications to these procedures might alter the performance as CE-IVD and have to be validated by the user. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.

9. Interfering substances

Red blood cells present in the specimen might exhibit autofluorescence which hinders signal recognition.

The following fixatives are incompatible with FISH:

- Bouin's fixative
- B5 fixative
- Acidic fixatives (e.g., picric acid)
- Zenker's fixative
- Alcohols (when used alone)
- Mercuric chloride
- Formaldehyde/zinc fixative
- Hollande's fixative
- Non-buffered formalin

10. Preparation of specimens

Recommendations:

- Fixation in 10% neutrally buffered formalin for 24 h at room temperature (18-25°C).
- Sample size ≤ 0.5 cm^3.
- Use premium quality paraffin.
- Embedding should be carried out at temperatures lower than 65°C.
- Prepare 2-4 µm microtome sections.
- Use positively charged microscope slides.
- Fix for 2-16 h at 50-60°C.

11. Preparatory treatment of the device

No reconstitution, mixing, or dilution is required. Bring probe to room temperature (18-25°C) before use, protect from light. Prior to opening the vial, mix by vortexing and shake down briefly.

12. Assay procedure

The Zyto*Mation* BCL2 Dual Color Break Apart FISH Probe is intended to be used on fully automated staining systems in combination with the respective FISH kits and FISH protocols. Please refer to the respective instructions for use of the system used for further information.

Specimen pretreatment
Perform specimen pretreatment (dewaxing, proteolysis) according to the respective instructions for use of the fully automated staining system.

Depending on the specimen, adjustments to the protocol might be needed. A validation of protocols deviating from recommended protocols must be performed by the user.

Denaturation and hybridization
For enzyme digestion and heat pretreatment, choose a protocol according to conditions prevalidated by the user depending on the specimen.

1. Set the denaturation of the specimens to 20 min at 75°C.

For the denaturation protocol, create a new protocol as described in the respective instructions for use of the automated Bond-MAX system.

2. Set the hybridization of the specimens to 2 h at 45°C.

For the hybridization protocol, create a new protocol as described in the respective instructions for use of the automated Bond-MAX system.

3. Load slides, FISH probe, enzyme dilution, and the BOND FISH Kit onto the system according to the instructions for use.
4. When the staining run is complete, remove the slides from the instrument. Protect slides from light.
5. Dehydrate slides with 70%, 90%, and 100% ethanol each for 1 min.
6. Air dry samples in the dark.
7. Pipette 25 µl DAPI/DuraTect-Solution (**MT7**) onto the slides. Avoiding trapped bubbles, cover the samples with a coverslip (24 mm x 60 mm). Incubate in the dark for 15 min.

Using a pipette tip which has been cut off to increase the size of the opening, can make the pipetting process easier. Avoid long exposure to light.

8. Store the slide in the dark. For longer storage periods, this should take place at 2-8°C.
9. Evaluation of the sample material is carried out by fluorescence microscopy.

13. Interpretation of results

With the use of appropriate filter sets, the hybridization signals of the probe appear green (proximal to the BCL2 breakpoint region) and orange (distal to the BCL2 breakpoint region).

Normal situation: In interphases of normal cells or cells without a translocation involving the BCL2 gene region, two green/orange fusion signals appear (see Fig. 2).

Aberrant situation: One BCL2 gene region affected by a translocation is indicated by one separate green signal and one separate orange signal (see Fig. 2).

Overlapping signals may appear as yellow signals.

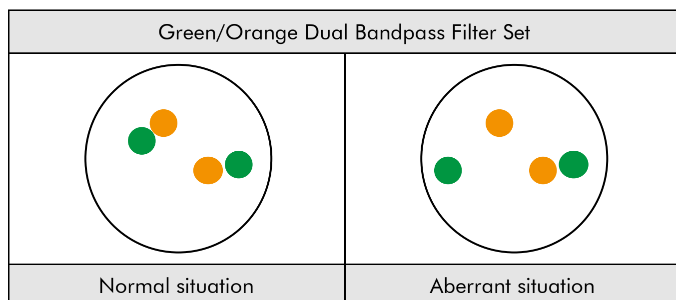

Fig. 2: Expected results in normal and aberrant interphase nuclei

Genomic aberrations due to small deletions, duplications or inversions might result in inconspicuous signal patterns.
Other signal distribution may be observed in some abnormal samples which might result in a different signal pattern than described above, indicating variant rearrangements. Unexpected signal patterns should be further investigated.

Please note:

- Due to decondensed chromatin, single FISH signals can appear as small signal clusters. Thus, two or three signals of the same size, separated by a distance ≤ 1 signal diameter, should be counted as one signal.
- Do not evaluate overlapping nuclei.
- Do not count over-digested nuclei (recognized by dark areas visible inside of the nuclei).
- Do not count nuclei with strong auto-fluorescence, which hinders signal recognition.
- A negative or unspecific result can be caused by multiple factors (see chapter 17).
- In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

14. Recommended quality control procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by internal and external controls. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

Internal control: Non-neoplastic cells within the specimen that exhibit normal signal pattern, e.g., fibroblasts.

External control: Validated positive and negative control specimens.

15. Performance characteristics

The performance of the probe was determined by comparison against the corresponding IVD approved FISH probe. The concordance was 100%.

Accuracy: The accuracy was calculated as 100%.

Analytical sensitivity: The analytical sensitivity was calculated as 100%.

Analytical specificity: The analytical specificity was calculated as 100%.

16. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

17. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all.

Weak signals or no signals at all

Possible cause	Action
No target sequences available	Use appropriate controls
Cell or tissue sample not fixed properly	Optimize fixing time and fixative
Proteolytic pretreatment not carried out properly	Optimize enzyme concentration and incubation time, increase or decrease if necessary
Old dehydration solutions	Prepare fresh dehydration solutions
Fluorescence microscope adjusted wrongly	Adjust correctly
Inappropriate filter sets used	Use filter sets appropriate for the fluochromes of the probe. *Triple-bandpass filter sets provide less light compared to single or dual-bandpass filter sets. Consequently, the signals may appear fainter using these triple-bandpass filter sets*
Photo-damage of the probes/fluorophores	Accomplish hybridization and washing steps in the dark

Cross hybridization signals; noisy background

Possible cause	Action
Proteolytic pretreatment too strong	Reduce enzyme concentration or incubation time

Morphology degraded

Possible cause	Action
Cell or tissue sample not fixed properly	Optimize fixing time and fixative
Proteolytic pretreatment not carried out properly	Optimize enzyme concentration or incubation time, decrease if necessary
Heat pretreatment not carried out properly	Optimize heat pretreatment

Overlapping nuclei

Possible cause	Action
Inappropriate thickness of tissue sections	Prepare 2-4 µm microtome sections

Specimen floats off the slide

Possible cause	Action
Unsuitable slide coating	Use appropriate slides
Insufficient drying of the tissue section	Adjust the time to dry the tissues sufficiently before staining
Fixation in formalin that was not properly neutral buffered	Use appropriate neutral buffered formalin of high quality

Weak counterstain

Possible cause	Action
Low concentrated DAPI solution	Use DAPI/DuraTect-Solution (ultra) (Prod. No. MT-0008-0.8) instead
DAPI incubation time too short	Adjust DAPI incubation time

18. Literature

- Da Cunha Santos G, et al. (2011) *Cancer Cytopathol* 119: 254-62.
- Dyer MJ, et al. (1994) *Blood* 83: 3682-8.
- Gu K, et al. (2008) *Arch Pathol Lab Med* 132: 1355-61.
- Hockenbery D, et al. (1990) *Nature* 348: 334-6.
- Impera L, et al. (2008) *Oncogene* 27: 6187-90.
- Kievits T, et al. (1990) *Cytogenet Cell Genet* 53: 134-6.
- López-Guillermo A, et al. (1999) *Blood* 93: 3081-7.
- Nelson BP, et al. (2007) *Am J Clin Pathol* 128: 323-32.
- Tibiletti MG, et al. (2009) *Hum Pathol* 40: 645-52.
- Tomita N, et al. (2009) *Haematologica* 94: 935-43.
- Weinberg OK, et al. (2007) *J Mol Diagn* 9: 530-7.
- Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992) ISBN 0 19 963327 4.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com

ZytoVision GmbH
Fischkai 1
27572 Bremerhaven/ Germany
Phone: +49 471 4832-300
Fax: +49 471 4832-509
www.zytovision.com
Email: info@zytovision.com

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