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

4250380P392RK

CE 0124

IVD

In vitro diagnostic medical device

according to IVDR (EU) 2017/746

1. Intended purpose

The ZytoMation 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, such as B-cell lymphoma, 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.

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

The probe is intended to be used as an aid to the differential diagnosis of B-cell lymphoma and therapeutic measures should not be initiated based on the test result alone.

2. 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.

3. Reagents provided

The ZytoMation 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

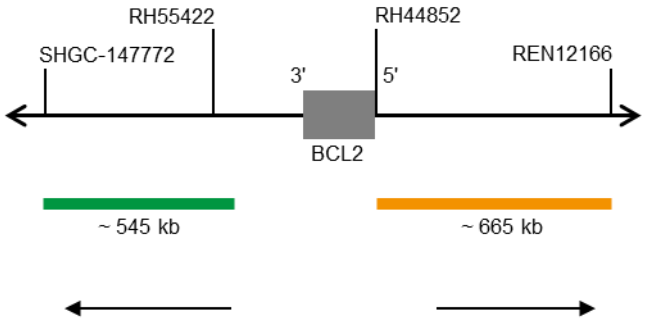

Fig. 1: BCL2 Probe map (not to scale)

The ZytoMation 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)

4. Materials required but not provided

- Bond-MAX or Bond-III system by Leica Biosystems
- Bond FISH Kit (DS9636)
- Bond Epitope Retrieval Solution 2 (AR9640)
- Bond Enzyme Pretreatment Kit (AR9551)
- DAPI/DuraTect-Solution (MT-0007-0.8)
- Bond Universal Covertiles (S21.4611)
- Bond Titration Kit (OPT9049)
- Dewax Solution (AR9222)
- Bond Wash Solution 10X Concentrate (AR9590)
- 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.

5. 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.

6. Warnings and precautions

- Read the instructions 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)!

- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- If reagents come into contact with skin, rinse skin immediately with copious amounts of water!
- A material safety data sheet is available on request for the professional user.
- Do not reuse reagents, unless reuse is explicitly permitted!
- 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.

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For use on the fully automated Bond-MAX or Bond-III system (Leica) 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/human geneticist 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/human geneticist 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 chapter 3. "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.

8. 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

9. 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.

10. 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.

11. Assay procedure

The Zyto*Mation* BCL2 Dual Color Break Apart FISH Probe is intended to be used on the fully automated Bond-MAX or Bond-III system 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.

11.1 Slide Setup on the fully automated Bond-MAX or Bond-III system

Set the following protocol steps in the Slide Setup Menu:

Staining:	*FISH Protocol D
Preparation:	*Dewax
HIER:	set up as described in step 1 below
Enzyme:	set up as described in step 2 below
Denaturation:	*Denaturation (10min)
Hybridization:	*ISH Hybridization (12Hr)

Specimen pretreatment

Perform specimen pretreatment (dewaxing, proteolysis, HIER) 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.

1. Pretreat specimens with the Bond Epitope Retrieval Solution 2 for 25 min at 100°C.

For the HIER protocol, create a new protocol as described in the respective instructions for use of the automated Bond-MAX/Bond-III system. Select the protocol for protocol step "HIER" in Slide Setup.

2. Pretreat specimens with BOND Enzyme Dilution at 37°C.

For enzyme digestion, choose a protocol according to conditions pre-validated by the user depending on the specimen and the conditions for HIER, denaturation and hybridization. Select the protocol for protocol step "Enzyme" in Slide Setup.

Denaturation and hybridization

1. Set the denaturation of the specimens to 10 min at 95°C.

*Select the predefined protocol "*Denaturation (10min)" for protocol step "Denaturation" in Slide Setup.*

2. Set the hybridization of the specimens to 12 h at 37°C.

*Select the predefined protocol "*ISH Hybridization (12Hr)" for protocol step "Hybridization" in Slide Setup.*

11.2 Staining run

1. Load slides, FISH probe, enzyme dilution, and the BOND FISH Kit onto the system according to the instructions for use.
2. When the staining run is complete, remove the slides from the instrument. Protect slides from light.

11.3 Post-hybridization and detection

1. Dehydrate slides with 70%, 90%, and 100% ethanol each for 1 min.
2. Air dry samples in the dark.
3. Pipette 20 µl DAPI/DuraTect-Solution (**MT7**) onto the slides. Avoiding trapped bubbles, cover the samples with a coverslip (24 mm x 50 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.

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

12. 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.

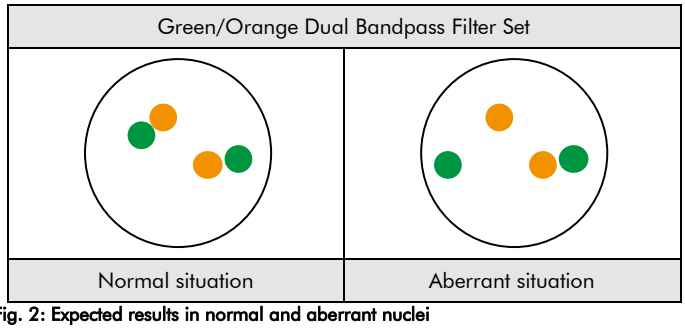

Fig. 2: Expected results in normal and aberrant nuclei

Genomic aberrations due to small deletions, duplications or inversions might result in inconspicuous signal patterns. Other signal patterns than those described above may be observed in some abnormal samples. These 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 16 "Troubleshooting").
- 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.

13. 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.

14. Performance characteristics

14.1 Analytical performance

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

Analytical sensitivity:	100% (95% CI 98.5 – 100.0)
Analytical specificity:	100% (95% CI 97.0 – 100.0)

14.2 Clinical performance

Diagnostic sensitivity:	100% (95% CI 98.0 – 100.0) vs. FlexISH BCL2/BCL6 DistinguISH Probe
Diagnostic specificity:	100% (95% CI 98.0 – 100.0) vs. FlexISH BCL2/BCL6 DistinguISH Probe

15. Disposal

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

16. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Please refer to www.zytovision.com for more information.

Weak signals or no signals at all

Possible cause	Action
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
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*

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 has not been 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
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

17. Literature

- Marino F, et al. (2021) *Virchows Archiv 479*: 565-573.
- Kievits T, et al. (1990) *Cytogenet Cell Genet 53*: 134-6.
- Willenbacher E, et al. (2020) *Annals of Hematology 99*: 2123-2132.
- Wilkinson DG: In Situ Hybridization, A Practical Approach, *Oxford University Press* (1992) ISBN 0 19 963327 4.

18. Revision

www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com
For the summary of safety and performance, please refer to www.zytovision.com.

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