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MOLECULAR DIAGNOSTICS

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BCL2 oligomix Alert kit

reagent for DNA "nested" amplification

REF **BANG08-02** CE -20°C

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INTENDED USE

«BCL2 oligomix Alert kit» is a qualitative assay for amplification of nucleic acids for the **detection of the DNA of the BCL2-IgH rearrangement, t(14;18) translocation, variants MBR and mcr** in DNA extracted from samples of peripheral blood collected in EDTA, medullary blood collected in EDTA or sodium citrate, and fresh and frozen biopsy samples.

The product is intended for use, alongside clinical data and other laboratory tests, in the diagnosis and monitoring of minimum residual disease in patients with follicular lymphoma and large cell.

ASSAY PRINCIPLE

The procedure involves execution of three types of amplification reaction, MBR, mcr and ABL, with a programmable heater (thermal cycler).

The MBR reaction involves two consecutive amplification reactions (nested) specific for the BCL2-IgH rearrangement, variant MBR (MBR).

The mcr reaction involves two consecutive amplification reactions (nested) specific for the BCL2-IgH rearrangement, variant mcr (mcr).

The ABL reaction involves only one single-round amplification reaction specific for the ABL gene (ABL) which is used as a control of sample suitability.

In the case of the MBR and mcr reactions the first amplification reaction is carried out specific for a region of the BCL2-IgH rearrangement and takes place in the first test tube using the DNA extracted from the test samples. The second amplification reaction is then carried out specific for the rearrangement and takes place in the second test tube using the product of the first amplification reaction.

The presence of the specific products of the second amplification reaction means that the DNA of the BCL2-IgH rearrangement is present in the starting sample.

In the case of the ABL reaction, the amplification reaction is carried out specific for a region of the ABL region and takes place in the test tube using the product of the first amplification reaction.

The presence of the specific product of the amplification reaction means that DNA of the ABL gene is present in the starting sample.

KIT DESCRIPTION

The kit provides the following components:

MBR OligoMIX and mcr OligoMIX for first amplification

Optimised mixtures of reagents for amplification of nucleic acids in a stabilizing solution, **aliquoted into ready-to-use «*monotest*» test tubes**. Each test tube contains 40 µL of solution and 50 µL of Vaseline.

MBR OligoMIX is aliquoted into **BLUE *«monotest»* test tubes**.

mcr OligoMIX is aliquoted into **NEUTRAL *«monotest»* test tubes**.

The reagent mixtures provide the primer oligonucleotides for the first amplification, the buffer system, magnesium chloride and triphosphate nucleotides.

In the case of **MBR OligoMIX**, the primer oligonucleotides are specific for the **MBR** and **IgH** gene regions.

In the case of the **mcr OligoMIX**, the primer oligonucleotides are specific for the **mcr** and **IgH** gene regions.

MBR OligoMIX and mcr OligoMIX for second amplification

Optimised mixtures of reagents for amplification of nucleic acids in a stabilizing solution, **aliquoted into ready-to-use «*monotest*» test tubes**. Each test tube contains 44 µL of solution and 50 µL of Vaseline.

MBR OligoMIX is aliquoted into **RED *«monotest»* test tubes**.

mcr OligoMIX is aliquoted into **GREEN *«monotest»* test tubes**.

The reagent mixtures provide the primer oligonucleotides for the second amplification, the buffer system, magnesium chloride and triphosphate nucleotides.

In the case of **MBR OligoMIX**, the primer oligonucleotides are specific for the **MBR** and **IgH** gene regions.

In the case of the **mcr OligoMIX**, the primer oligonucleotides are specific for the **mcr** and **IgH** gene regions.

ABL OligoMIX for control amplification

An optimised mixture of reagents for amplification of nucleic acids in a stabilizing solution, **aliquoted into ready-to-use YELLOW «*monotest*» test tubes**. Each test tube contains 40 µL of solution and 50 µL of Vaseline.

The reagent mixture provides the primer oligonucleotides for the control amplification, the buffer system, magnesium chloride and triphosphate nucleotides.
The primer oligonucleotides are specific for the **ABL** gene.

The kit enables **25 reactions**, including positive and negative controls.

MATERIALS PROVIDED IN THE KIT

Component	Description	Quantity	Composition	Labelling
First amplification MBR OligoMIX	amplification mixture in **0.2 mL BLUE test tubes**	**25 x 90 µL**	External oligonucleotides, potassium chloride, TRIS base, TRIS hydrochloride, Triton X-100, magnesium chloride, Deoxyribonucleotide triphosphates, Vaseline	-
First amplification mcr OligoMIX	amplification mixture in **0.2 mL NEUTRAL test tubes**	**25 x 90 µL**	External oligonucleotides, potassium chloride, TRIS base, TRIS hydrochloride, Triton X-100, magnesium chloride, Deoxyribonucleotide triphosphates, Vaseline	-
ABL OligoMIX for control amplification	amplification mixture in **0.2 mL YELLOW test tubes**	**25 x 90 µL**	Oligonucleotides, potassium chloride, TRIS base, TRIS hydrochloride, Triton X-100, magnesium chloride, Deoxyribonucleotide triphosphates, Vaseline	-
Second amplification MBR OligoMIX	amplification mixture in **0.2 mL RED test tubes**	**25 x 94 µL**	Internal oligonucleotides, potassium chloride, TRIS base, TRIS hydrochloride, Triton X-100, magnesium chloride, Deoxyribonucleotide triphosphates, Vaseline	-
Second amplification mcr OligoMIX	amplification mixture in **0.2 mL GREEN test tubes**	**25 x 94 µL**	Internal oligonucleotides, potassium chloride, TRIS base, TRIS hydrochloride, Triton X-100, magnesium chloride, Deoxyribonucleotide triphosphates, Vaseline	-

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MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

- Laminar airflow hood.
- Disposable latex powder-free gloves or similar material.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Sterile micropipettes and tips with aerosol filter or positive displacement (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL).
- Sterile bidistilled water.
- Programmable heater (thermal cycler).

OTHER PRODUCTS REQUIRED

The reagents for DNA extraction from the test samples, for the positive amplification control and for detection of amplified DNA are **not** included in this kit. To perform these analytical steps the following products, manufactured by ELITechGroup S.p.A., are recommended:

«EXTRAcell» (code EXTD02), kit for DNA extraction from cellular samples; the kit enables 50 extractions.

«EXTRAffin» (code EXTF01), kit for extraction of nucleic acids from biopsy samples; the kit enables 50 extractions.

«BCL2 - Positive Control» (code CTRG08), positive amplification control of plasmid DNA; the kit enables 25 sessions.

«DNA polymerase 2U / µL» (code ER40 and ER140), thermostable DNA polymerase enzyme for amplification of nucleic acids; the kits provide 125 reactions.

«ELECTROPHORESIS 3» (code EPH03), detection of amplified DNA for electrophoresis on agarose gel; the kit provides 120 detections.

WARNINGS AND PRECAUTIONS

This kit is exclusively for *in vitro* use.

Warnings and general precautions

Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121°C for one hour before disposal.

Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothing and gloves and protect eyes and face.

Never pipette solutions by mouth.

Do not eat, drink, smoke or apply cosmetic products in the work areas.

Wash hands carefully after handling samples and reagents.

Dispose of leftover reagents and waste in compliance with regulations in force.

Read all the instructions provided with the kit before running the assay.

Follow the instructions provided with the kit while running the assay.

Do not use the kit after the expiry date.

Only use the reagents provided in the kit and those recommended by the manufacturer.

Do not mix reagents from different batches.

Do not use reagents from other manufacturers' kits.

Warnings and precautions for molecular biology

Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.

It is necessary to have separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never introduce an amplification product in the area designed for extraction / preparation of amplification reactions.

It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification / detection of amplification products to the area designed for the extraction / preparation of the amplification reactions.

The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar flow hood. Test tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.

Reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.

Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components

The test tubes containing **OligoMIX** for the **first, second** and **control amplifications** are disposable and therefore must be used once only in amplification reactions.

OligoMIX for the **first, second** and **control amplifications** carries the following safety warnings (S):

S 23-25. Do not breathe gas/fumes/vapour/spray. Avoid contact with eyes.

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SAMPLES AND CONTROLS

Samples

This product must be used with **DNA** extracted from the following biological samples: peripheral blood collected in EDTA, medullary blood collected in EDTA or sodium citrate, fresh or frozen biopsy samples.

Peripheral blood collected in EDTA and medullary blood collected in EDTA or sodium citrate

The peripheral blood in EDTA or medullary blood in EDTA or sodium citrated that are to be used in DNA extraction must be collected according to laboratory guidelines, transported at +2° / +8°C and sto red at +2° / +8°C for a maximum of three days.

Do not freeze peripheral or medullary blood in order to prevent loss of DNA.

Instructions for DNA extraction are contained in the instruction manual for **«EXTRAcell»**.

Fresh or frozen biopsy samples

The fresh biopsy samples to be used for DNA extraction must be collected according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of four hours, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods.

It is advisable to split the samples that are to be stored frozen into fragments of c. 1 mm^3 in order to prevent repeated cycles of freezing and thawing.

Instructions for DNA extraction are contained in the instruction manual for **«EXTRAffin»**.

Interfering substances

The DNA extracted from the starting sample must not contain heparin or haemoglobin in order to prevent the problem of inhibition and the possibility of frequent invalid results.

There are no data available concerning inhibition caused by antibiotics, antiviral drugs, chemotherapeutic drugs or immunosuppressants.

Amplification controls

It is absolutely mandatory to validate each amplification session with a positive control reaction and a negative control reaction.

For the negative control, use sterile bidistilled water (not supplied with kit) added to the reaction in place of the DNA extracted from the sample.

For the positive control, use the DNA extracted from a sample that has already tested positive, or **«BCL2 - Positive Control».**

Quality controls

It is recommended to validate the whole analysis procedure of each extraction and amplification session by processing an already been tested negative and positive sample or a calibrated reference material.

PROCEDURE

Setting the thermal cycle
(To be performed in the amplification / detection area of the amplification products)

Before starting the session it is important to do the following:
- Make sure that the thermal block of the programmable heater (thermal cycler) is compatible with the format of the ***«monotest»*** **test tubes** supplied with the kit (0.2 mL test tubes);
- Referring to the instrument documentation, check the procedure for controlling the temperature used by the thermal cycler for the thermal cycle;
- When possible, select **temperature regulation directly on the thermal block** (e.g. thermal cycler Hybaid™ or Eppendorf™);
- Where this is not possible (e.g. for GeneAmp® PCR System thermal cycler by Applied Biosystems™) use the preset temperature regulation system;
- When requested, set a reaction volume of **100 µL** on the thermal cycler;
- Set the parameters of the thermal cycle on the thermal cycler as shown in the following tables.

First amplification thermal cycle		
Number of cycles	**Temperature**	**Timing**
1 cycle	95° C	4 min.
35 cycles	95° C	1 min.
	60° C	1 min.
	72° C	1 min.
1 cycle	72° C	5 min.

Second amplification thermal cycle		
Number of cycles	**Temperature**	**Timing**
1 cycle	95° C	2 min.
30 cycles	95° C	1 min.
	60° C	1 min.
	72° C	1 min.
1 cycle	72° C	5 min.

Preparation of the first amplification
(To be performed in the extraction / preparation area of the amplification reaction)

Each sample requires a tube. The kit enables 25 reactions MBR, 25 reactions mcr, 25 reactions ABL, including positive and negative controls. The minimum number of reactions per session is nine: three MBR reactions (positive control, negative control and test sample), three mcr reactions (same as first), three ABL reactions (same as first).

Before starting the session it is important to do the following:
- Thaw the test samples and the **«BCL2 - Positive Control»,** centrifuge for 5 seconds to bring the contents to the bottom and keep in ice;
- Thaw a **BLUE (MBR)** ***«monotest»*** **test tube** for each sample that is to be analysed, one for the negative control and one for the positive control; centrifuge* for 5 seconds to bring the contents to the bottom and mark clearly using a permanent marker pen and keep in ice.
- Thaw a **NEUTRAL (mcr)** ***«monotest»*** **test tube** for each sample that is to be analysed, one for the negative control and one for the positive control; centrifuge* for 5 seconds to bring the contents to the bottom and mark clearly using a permanent marker pen and keep in ice.
- Thaw a **YELLOW (ABL)** ***«monotest»*** **test tube** for each sample that is to be analysed and one for the negative control; centrifuge* for 5 seconds to bring the contents to the bottom and mark clearly using a permanent marker pen and keep in ice.

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***N.B.:** The **«*monotest*» tubes** are "thin-walled" and must be handled with care to avoid breakage. Centrifugation must be carried out using reducers when necessary and according to the methods described in this instruction manual.

- Remove the **«DNA pol. 2U / µL»**, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep in ice;
- Dilute the **«DNA pol. 2U / µL»** enzyme with sterile bidistilled water as shown in the following table. Prepare enough diluted enzyme for all the reactions **MBR**, **mcr**, **ABL** (including controls) plus one extra as a safety margin. The diluted enzyme **cannot** be stored.

Number of reactions	DNA pol. 2U / µL	Water	Total volume
4	2.0 µL	18.0 µL	20 µL
5	2.5 µL	22.5 µL	25 µL
6	3.0 µL	27.0 µL	30 µL
7	3.5 µL	31.5 µL	35 µL
8	4.0 µL	36.0 µL	40 µL
9	4.5 µL	40.5 µL	45 µL
10	5.0 µL	45.0 µL	50 µL
11	5.5 µL	49.5 µL	55 µL
12	6.0 µL	54.0 µL	60 µL
13	6.5 µL	58.5 µL	65 µL
14	7.0 µL	63.0 µL	70 µL
15	7.5 µL	67.5 µL	75 µL
16	8.0 µL	72.0 µL	80 µL
17	8.5 µL	76.5 µL	85 µL
18	9.0 µL	81.0 µL	90 µL
19	9.5 µL	85.5 µL	95 µL
20	10.0 µL	90.0 µL	100 µL
21	10.5 µL	94.5 µL	105 µL

1. Add **5 µL** of diluted thermostable DNA polymerase (1 U) to each **BLUE (MBR), NEUTRAL (mcr)** and **YELLOW (ABL) «*monotest*» test tube**.
2. Add **5 µL** of DNA extract to each **BLUE, NEUTRAL** and **YELLOW «*monotest*» test tube**. Proceed in the same way for the negative and positive controls.
3. Transfer the **BLUE, NEUTRAL** and **YELLOW «*monotest*» test tubes** to the thermal cycler in the amplification / detection area of the amplification products and start the thermal cycle of the first amplification.

Preparation of the second amplification
(To be performed in the extraction / preparation area of the amplification reaction)

Before starting the session it is important to do the following:
- Remove the **«DNA pol. 2U / µL»**, centrifuge the tubes for 5 seconds to bring the contents to the bottom and keep in ice;
- Thaw the same number of **RED (MBR) «*monotest*» tubes** as blue ones; centrifuge* the tubes for 5 seconds to bring the contents to the bottom and mark clearly using a permanent marker pen and keep in ice.
- Thaw the same number of **GREEN (mcr) «*monotest*» tubes** as neutral ones; centrifuge* the tubes for 5 seconds to bring the contents to the bottom and mark clearly using a permanent marker pen and keep in ice.

***N.B.:** The **«*monotest*» tubes** are "thin-walled" and must be handled with care to avoid breakage. Centrifugation must be carried out using reducers when necessary and according to the methods described in this instruction manual.

- Dilute the **«DNA pol. 2U / µL»** enzyme with sterile bidistilled water as shown in the previous table. Prepare enough diluted enzyme for all the reactions **MBR** and **mcr** (including controls) plus one extra, as a safety margin. The diluted enzyme **cannot** be stored.

4. Add **5 µL** of diluted thermostable DNA polymerase (1 U) to each **RED (MBR)** and **GREEN (mcr) «*monotest*» test tube**.
5. Add **1 µL** of the product of the first amplification to each **RED «*monotest*» test tube (MBR)** from the corresponding **BLUE (MBR) «*monotest*» test tube**.
6. Add **1 µL** of the product of the first amplification to each **GREEN «*monotest*» test tube (mcr)** from the corresponding **NEUTRAL (mcr) «*monotest*» test tube**.

N.B.: The product of the first amplification can contaminate subsequent assays. Isolate the residual product of the first amplification and change gloves at the end of this phase of the procedure.

7. Transfer the **RED** and **GREEN «*monotest*» test tubes** to the thermal cycler in the amplification / detection area of the amplification products and start the thermal cycle of the second amplification.

N.B.: The reaction product may be stored at -20°C for a maximum of one month.

Detection of the specific amplification product
(To be performed in the amplification / detection area of the amplification products)

The specific product of the second amplification can be detected and identified by electrophoretic separation using a 4% agarose gel with 1 µg / mL ethidium bromide in TAE 1x buffer (20 mM TRIS base, 20 mM TRIS acetate, 1 mM EDTA disodium), as in **«ELECTROPHORESIS 3».**

The **specific products of the second MBR amplification** measures	**from 80 to 300 bp**.

The **specific products of the second mcr amplification** measure:	**from 500 to 1000 bp**

The **specific product of the ABL amplification** measures**:**	**715 bp**

N.B.: The product of the second amplification can contaminate subsequent assays. Isolate the residual product of the second amplification and product waste in the detection phase.

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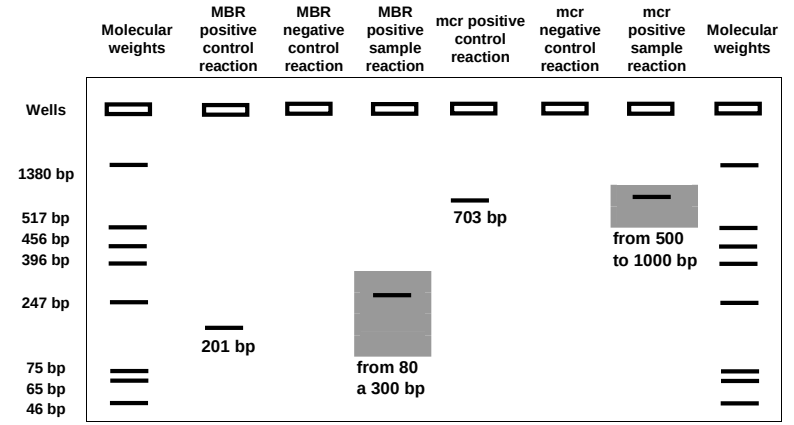
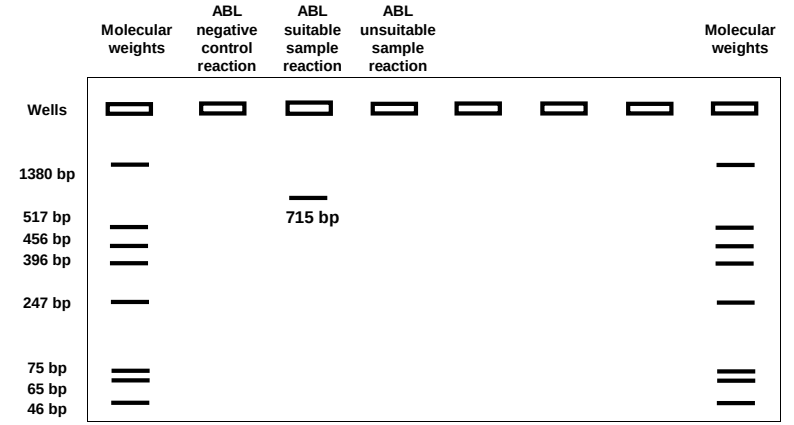
By way of example, below is a diagram representing the results of the electrophoretic separation of the products of the second MBR and mcr amplification.



N.B.: Owing to the homology sometimes present in the J region of the IgH gene, a further amplification product measuring c. 500 bp may be observed in association with the specific product of the second amplification for MBR or mcr.

By way of example, below is a diagram representing the results of the electrophoretic separation of the products of the ABL amplification.



It is absolutely mandatory to validate the identity of the product of the second amplification of a sample by comparing its migration to gel with the migration of a molecular weight marker, and with the migration of the product of the second amplification of the positive control.

The presence of non-specific products of different dimensions from the specific products of the second amplification of a sample has no significance for the detection of the DNA of the MBR and mcr rearrangements and the DNA of the ABL gene.

Interpreting the results

The results of the negative and positive control reactions are used to validate the amplification session as shown in the following tables:

Amplification of the negative controls Specific product MBR, mcr and ABL	Amplification
ABSENT	CORRECT

Amplification of the positive controls Specific product of MBR and mcr	Amplification
PRESENT	CORRECT

If the specific amplification product is present in one of the negative control reactions, this means that problems have occurred during the amplification phase (contamination), which may cause false positives. The session is invalid and must be repeated from the amplification phase.

If the specific amplification product is absent in one of the positive control reactions, this means that problems have occurred during the amplification phase (inefficient or absent amplification), which may cause false negatives. The session is invalid and must be repeated from the amplification phase.

This kit is able to detect a minimum of 10 copies of DNA of the MBR or mcr rearrangements per amplification reaction (detection limit of the product, see paragraph on Performance Characteristics on page 14).

The results of the reactions in the test samples are used to evaluate the presence of MBR and mcr DNA as described in the following tables:

Amplification of the sample		Sample suitability	Assay result	DNA of MBR or mcr
Specific product of MBR or mcr	Specific product ABL			
ABSENT	ABSENT	not suitable	invalid	-
	PRESENT	suitable	valid, negative	NOT DETECTED
PRESENT	ABSENT	suitable*	valid, positive	PRESENT
	PRESENT	suitable	valid, positive	PRESENT

If both the specific amplification product of MBR or mcr along with the specific amplification product of the ABL gene of the control amplification are absent in a sample reaction, this means that problems have occurred during the amplification phase (inefficient or absent amplification) or in the extraction phase (absence of DNA, presence of inhibitors or insufficient number of cells in the starting sample) which may cause false negatives. The sample is not suitable, the assay is invalid and must be repeated beginning with extraction of a new sample.

If the specific amplification product of MBR and mcr is absent from a sample reaction, while the specific amplification product of the ABL gene of the control amplification is present, this means that the DNA of the MBR and mcr rearrangements has not been detected in the DNA extracted from the sample, but it is not possible to exclude the presence of the DNA of the MBR and mcr rearrangements at a lower titre than the detection limit of the product (see paragraph on Performance Characteristics on page 14). In this case the result would be a false negative.

The results obtained with this product must be interpreted in consideration of all the clinical data and the other laboratory tests done on the patient.

***N.B.:** When the specific amplification product of the MBR or mcr rearrangements is present in a amplification reaction, while the specific product of the ABL gene of the control amplification is absent, this means that the sample is still suitable and the positive result of the assay is valid. In fact the amplification reaction of ABL gene is less efficient (single round) than the amplification reaction of MBR and mcr (nested).

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Calculating the detection limits

When a particular extraction method is used and referred to a particular unit of measurement, the detection limit may be calculated from the detection limit of the amplification reaction according to the following formula:

Detection limit = Fe x Ee x Fa x 10 copies DNA /reaction

Fe is the ratio between the reference unit of measurement and the sample used in the extraction, for example:

Extraction	unit of measurement	extraction sample	Fe
«**EXTRAcell**»	copies of DNA / 1,000,000 cells	500,000 cells	Fe = 10^6 c. / 5 x 10^5 c. = 2

Ee is the inverse of the extraction efficiency, for example:

Extraction	extraction efficiency	Ee
«**EXTRAcell**»	efficiency approx. 100%, i.e. 1.0	Ee = 1 / 1.0 = 1

Fa is the ratio between the volume of DNA extracted and the volume used in the amplification reaction, for example:

Extraction	volume of extract	volume in reaction	Fa
«**EXTRAcell**»	100 µL	5 µL	Fa = 100 µL / 5 µL = 20

When ELITechGroup S.p.A. extraction kits are used, the formula becomes:

Extraction	Detection limits
«**EXTRAcell**»	**Detection limit = 400 copies of DNA / 1,000,000 cells**

PROCEDURE LIMITATIONS

Use only DNA extracted from the following biological samples with this product: .peripheral blood collected in EDTA, medullary blood collected in EDTA or sodium citrate, fresh or frozen biopsy samples.

Do not use DNA extracted from heparinized samples with this product: heparin inhibits the amplification reaction of nucleic acids and causes invalid results.

Do not use DNA extract that is contaminated with haemoglobin with this product: these substances may inhibit the nucleic acid amplification reaction and cause invalid results.

There are no data available concerning inhibition caused by antibiotics, antiviral drugs, chemotherapeutic drugs or immunosuppressants.

The results obtained with this product are subject to the correct collection, transport, storage and preparation of samples. To avoid result errors it is therefore necessary to take particular care during these phases and to carefully follow the instructions provided with the products for nucleic acid extraction.

Owing to its high analytical sensitivity, the nested amplification assay of nucleic acids used in this product is subject to contamination from clinical samples positive for MBR and mcr, positive controls and the amplification reaction products themselves. Contamination leads to false positive results. The product has been designed in such a way as to reduce contamination; nevertheless, this phenomenon can only be prevented by following good laboratory practices and by complying scrupulously with the instructions provided in this manual.

This product must be handled by personnel trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of work clothes and premises that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product must be handled by personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid result errors.

It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results.

This product requires the use of special clothing and instruments for extraction/preparation of amplification reactions and for amplification/detection of amplification products to avoid false positive results.

A negative result obtained with this product suggests that the DNA of the MBR or mcr rearrangements was not detected in the DNA extracted from the sample, but it may also contain DNA of the MBR or mcr rearrangements at a lower titre than the detection limit for the product (see paragraph on Performance Characteristics on page 14); in this case the result would be a false negative.

As with any diagnostic device, the results obtained with this product must be interpreted in consideration of all the clinical data and other laboratory tests done on the patient.

As with any diagnostic device, there is a residual risk of obtaining invalid results, false positives and false negatives with this product. This residual risk cannot be eliminated or reduced any further. In particular situations, this residual risk can contribute to incorrect decisions with potentially grave consequences for the patient.

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PERFORMANCE CHARACTERISTICS

Analytical sensitivity: detection limit of MBR OligoMIX and mcr OligoMIX

The analytical sensitivity of this OligoMIX enables identification of approx. 10 target DNA molecules in 5 µL of DNA extract added to the amplification reaction.

In terms of the detection limit, the analytical sensitivity of the assay was determined using plasmid DNA containing the amplification product as the reference material, and whose initial concentration was measured by spectrophotometer (pMBR and pmcr).

The pMBR plasmid DNA was diluted to a titre of 10 copies / 5 µL and the dilution was used in two repeats in six different sessions, performing the nested amplification procedure with MBR OligoMIX by ELITechGroup S.p.A.
The final results are summed up in the following table.

Samples	No. sessions	No. repeats	MBR positives	MBR negative
10 copies pMBR	6	2	12	0

The pmcr plasmid DNA was diluted to a titre of 10 copies / 5 µL and the dilution was used in two repeats in six different sessions, performing the nested amplification procedure with mcr OligoMIX by ELITechGroup S.p.A.
The final results are summed up in the following table.

Samples	No. sessions	No. repeats	mcr positives	mcr negative
10 copies pmcr	6	2	12	0

Analytical sensitivity: detection limit of ABL OligoMIX

The analytical sensitivity of this OligoMIX enables identification of approx. 100 target DNA molecules in 5 µL of DNA extract added to the amplification reaction.

In terms of the detection limit, the analytical sensitivity of the assay was determined using human genomic DNA as reference material, whose initial concentration was measured by spectrophotometer.

The human genomic DNA was diluted to a titre of 5 ng / 5 µL and the dilution was used in two repeats in six different sessions, performing the single-round amplification procedure with ABL OligoMIX by ELITechGroup S.p.A.
The final results are summed up in the following table.

Samples	No. sessions	No. repeats	ABL positives	ABL negative
5 ng genomic DNA	6	2	12	0

Diagnostic sensitivity: detection efficiency on polymorphisms

The diagnostic sensitivity of the assay, that is the efficiency of detection on polymorphisms, was evaluated by comparison of sequences with nucleotide databases.

The alignment test of the regions chosen for hybridization of the **MBR OligoMIX** primer oligonucleotides for the MBR rearrangement with the sequences available in the database of the gene region of the BCL2-IgH rearrangement showed preservation and absence of significant mutations.

The alignment test of the regions chosen for hybridization of the **mcr OligoMIX** primer oligonucleotides for the mcr rearrangement with the sequences available in the database of the gene regions of the BCL2-IgH rearrangement showed preservation and absence of significant mutations.

The alignment test of the regions chosen for hybridization of the **ABL OligoMIX** primer oligonucleotides for the ABL gene with the sequences available in the database of the gene region codifying ABL showed preservation and absence of significant mutations.

Diagnostic specificity: negative samples

The diagnostic specificity of the assay, confirming negative samples, was tested by analysing a number of normal human genomic DNA samples.

The diagnostic specificity was evaluated using the reference material of human genomic DNA that was negative for the MBR or mcr rearrangements, whose initial concentration was measured by spectrophotometer.

The human genomic DNA was diluted to a concentration of 500 ng / 5 µL and the dilution was used in two repeats in six different sessions, performing the nested amplification procedure with MBR OligoMIX by ELITechGroup S.p.A.
The final results are summed up in the following table.

Samples	No. sessions	No. repeats	MBR positives	MBR negative
500 ng genomic DNA	6	2	0	12

The human genomic DNA was diluted to a concentration of 500 ng / 5 µL and the dilution was used in two repeats in six different sessions, performing the nested amplification procedure with mcr OligoMIX by ELITechGroup S.p.A.
The final results are summed up in the following table.

Samples	No. sessions	No. repeats	mcr positives	mcr negative
500 ng genomic DNA	6	2	0	12

Analytical specificity: potential interference markers

The analytical specificity of the assay, that is the cross-reactivity with other potential interference markers, was evaluated by comparison of sequences with nucleotide databases.

The alignment test of the regions chosen for hybridization of the **MBR OligoMIX** primer oligonucleotides for the MBR rearrangement with the sequences available in a database of human genomic regions other than the MBR and IgH gene regions, including the regions of the complete human genome, showed the specificity of the amplification system.

The alignment test of the regions chosen for hybridization of the **mcr OligoMIX** primer oligonucleotides for the mcr rearrangement with the sequences available in a database of human genomic regions other than the mcr and IgH gene regions, including the regions of the complete human genome, showed the specificity of the amplification system.

The alignment test of the regions chosen for hybridization of the **ABL OligoMIX** primer oligonucleotides for the ABL gene with the sequences available in a database of human genomic regions other than the ABL human gene, including the regions of the complete human genome, showed the specificity of the amplification system.

N.B.: The complete data and results of the tests carried out to evaluate the performance characteristics of the product are recorded in Section 7 of the Product Technical File "BCL2 oligomix Alert kit", FTP BANG08.

REFERENCES

J. G. Gribben et al. (1994) *Blood* 12: 3800 - 3807

BCL2 oligomix Alert kit
reagent for DNA "nested" amplification

REF **BANG08-02**

TROUBLESHOOTING

Specific amplification product present in the negative control reaction

Possible causes	Solutions
Error displacing the DNA extract.	Open one test tube at a time; avoid spilling the contents of the test tube; always change tips.
Error displacing the first amplification product.	Open one test tube at a time; avoid spilling the contents of the test tube; always change tips.
Contamination of the reagents prepared for the session.	Take great care when diluting and displacing the enzyme; always change tips.
Contamination of the sterile water for enzyme dilution.	Use a new aliquot of sterile water.
Contamination of the enzyme stock.	Use a new aliquot of enzyme.
Contamination of the extraction / preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

Specific amplification product absent in the Positive Control reaction

Possible causes	Solutions
Enzyme too diluted or error displacing enzyme.	Check the dilution calculations; take great care when displacing the enzyme and mix properly.
Error displacing the positive control.	Take great care when displacing the positive control.
Degradation of the positive control.	Use a new aliquot of positive control.
Thermal cycle setting error.	Check the thermal cycle settings on the thermal cycler.
Error displacing the first amplification product.	Carefully remove and dispense the product of the first amplification into the second amplification.
Error displacing the second amplification product in the gel.	Take care when loading the product of the second amplification in the gel.

Non-specific amplification products present in the sample reactions

Possible causes	Solutions
Enzyme too concentrated.	Check the calculations of the enzyme dilution.
Preparation times of the first amplification reaction are too long.	Keep the test tubes to which DNA extract have already been added in ice until transferred to the thermal cycler.
Thermal cycle setting error.	Check the thermal cycle settings on the thermal cycler.
Excess DNA extract in the reaction.	Assess the concentration of the DNA extract; do not add more than 1 µg of DNA per reaction.

Specific amplification product absent in the ABL sample control reaction

Possible causes	Solutions
Heparin present in the whole blood sample	The blood sample must use EDTA or sodium citrate as anticoagulants.
Error storing blood sample.	Blood must be treated for DNA extraction within a few hours and must not be frozen.
Error during extraction.	Check extraction operations. Take care when preparing and performing extraction and follow instructions closely.
Degradation of extracted sample.	DNA must be extracted with materials and reagents containing no DNAses. DNA extract must be stored at -20°C or at -70°C.
Error during amplification.	See suggestions in paragraph “Specific amplification product absent in the positive control reaction”.

BCL2 oligomix Alert kit
reagent for DNA "nested" amplification

REF **BANG08-02**

SYMBOLS

REF	Catalogue number.
	Upper temperature limit.
LOT	Batch code.
	Use by (last day of month).
IVD	*In vitro* diagnostic medical device.
CE	In keeping with the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical devices.
	Contents sufficient for "N" tests.
CONT	Contents.
	Please refer to the instructions for use.
	Manufacturer.