ELITechGroup
EMPOWERING IVD

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INFORMATIVE NOTICE dated 11/10/2024

IMPORTANT FOR PRODUCT USERS

«PHILADELPHIA P210 RNA Reference»
code number SPG07-210
lot number U0724-145

This informative notice is necessary for the final data interpretation, for data check and eventually for the calculation of the Conversion Factor (CF) specific for each single laboratory.

The Conversion Factor (CF) is required to obtain a value aligned to the International Scale (IS) for the quantitative dosing of BCR-ABL P210 cDNA using Real Time PCR amplification assay.

IMPORTANT

This informative notice is valid for the lot number **U0724-145** of «**PHILADELPHIA P210 RNA Reference**» product, code n° SPG07-210, for the packaging lot starting from 11/10/2024.

From this batch, the product provides 5 different mixtures of P210 b3a2 total RNA, corresponding to dilutions 10^{-1},10^{-2},10^{-3}, 10^{-4} and $10^{-4.5}$. The product characteristics can be found in the instruction for use manual SCH mSPG07-210_en, Review 08.

Please contact the ELITechGroup staff at the following e-mail address: emd.ifu@elitechgroup.com, to request the manual for a previous version of the product.

Ideally «**PHILADELPHIA P210 RNA Reference**» product should be reverse-transcribed and amplified **at least in duplicate** every Real Time PCR quantitative analytical session. The minimum recommended use of the product is recommended at least **every 3 months** and every time a change in the CML monitoring procedure occurs (for instance: Real Time instrument calibration, change of reagents, different operator, …).

The table below reports the percentage of mRNA with the translocation t(9;22) compared to the mRNA of ABL present in each tube of a mixture of **P210 b3a2** RNA:

Tube	**P210 b3a2 10^{-1}**	**P210 b3a2 10^{-2}**	**P210 b3a2 10^{-3}**	**P210 b3a2 10^{-4}**	**P210 b3a2 $10^{-4.5}$**
Insert Color	**RED**	**BLUE**	**GREEN**	**YELLOW**	**VIOLET**
Reference Titre *	**29.3971%**	**3.2031%**	**0.3665%**	**0.0316%**	**0.0088%**

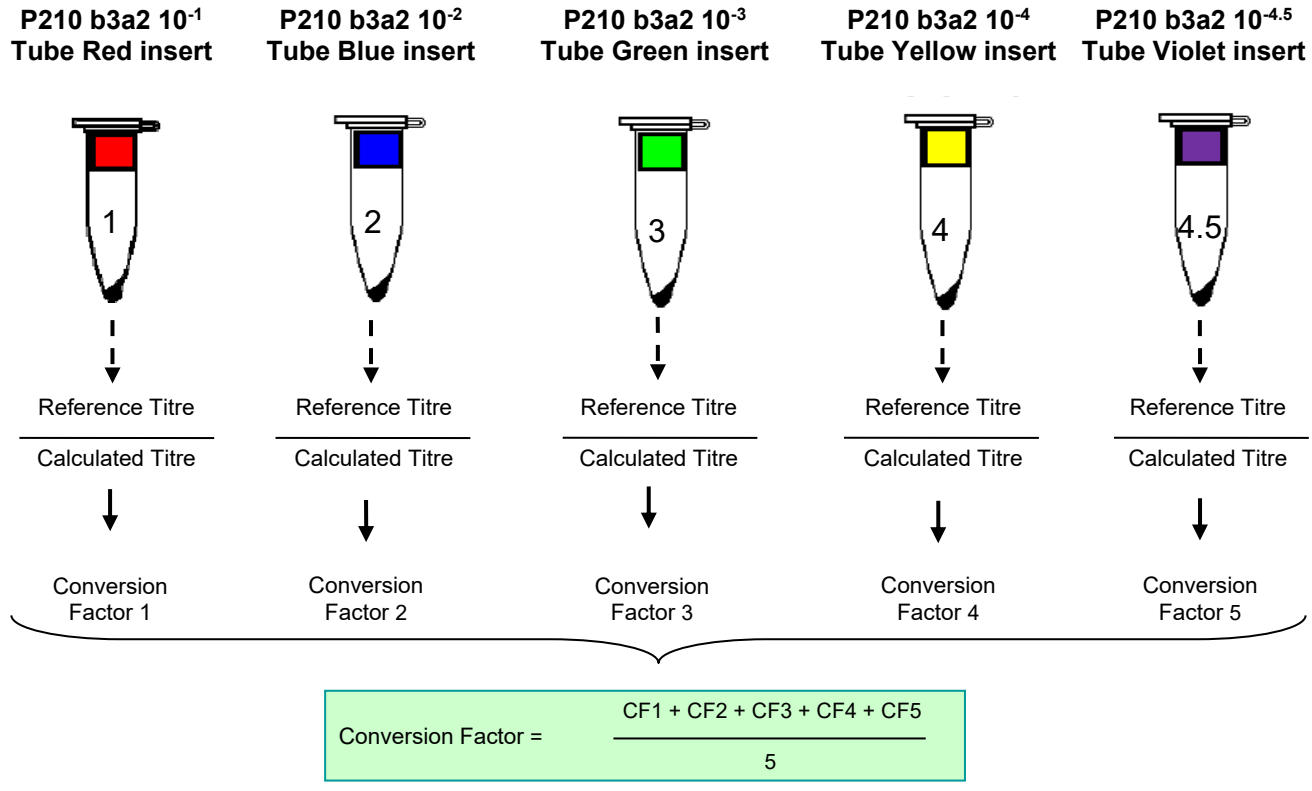
* The **RNA REFERENCE TITRE** of the lot **U0724-145** of **«PHILADELPHIA P210 RNA Reference»** product has been assigned as a result of a calibration performed by using a Conversion Factor validated by the REFERENCES LABORATORIES AFFECTING THE LABNET ITALIAN PROJECT, with the "1st World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR" (NIBSC, UK, code: 09/138), according to instructions provided by NIBSC *http://www.nibsc.ac.uk/documents/ifu/09-138.pdf* and in the article *HE White et al. Blood 2010, Supplementary Methods B*.

Each mixture of **P210 b3a2 RNA** of **«PHILADELPHIA P210 RNA Reference»** product will undergo reverse-transcription and amplification with the current system used in the laboratory.

For every mixture of **P210 b3a2 RNA** a Calculated Titre will be obtained (mean value of the replicates of each aliquot).

It is suggested to use P210 b3a2 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and $10^{-4.5}$ mixtures to calculate the International Unit Conversion Factor.

The Conversion Factor is obtained by the mean value of the ratios between each Reference Titre and the corresponding Calculated Titre for each mixture of **P210 b3a2 RNA**.



REAL-TIME Alert Q-PCR

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PHILADELPHIA P210 RNA Reference

total RNA control for quantitative assay

REF SPG07-210 CE -70°C

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INTENDED USE

The **«PHILADELPHIA P210 RNA Reference»** product is intended for use as reference RNA to evaluate the performances of quantitative nucleic acids amplification assays for the **detection of the cDNA of the BCR-ABL rearrangement, t(9;22) translocation, *Philadelphia* chromosome, variant P210 and its quantification normalized in comparison with the cDNA of the ABL gene.**

PRODUCT DESCRIPTION

The product supplies four different mixtures of total RNA **P210 b3a2** extracted from two human cell lines to the final concentration of 400 ng / µL. Each solution is obtained by dilution of known quantity of total RNA from a cell line positive for the t(9;22) translocation, with BCR-ABL rearrangement variant P210 b3a2, into total RNA of a normal cell line*.

The product supplies 18 µL of each of the five dilutions, aliquoted into five test tubes with different coloured inserts.

The product is sufficient to perform **6 separate analytical sessions**, using 2.5 µL of sample (equivalent to 1 µg of total RNA) in the reverse transcription reaction with the product "**RT Kit plus**" (ELITechGroup S.p.A., code BRK200).

The product is sufficient to perform **5 separate analytical sessions**, using 0.75 µL (equivalent to 300 ng of total RNA) in the reverse transcription and real time amplification reaction (one-step method) with the product "**BCR-ABL P210 Elite MGB® Kit**" (ELITechGroup S.p.A., code RTSG07PLD210).

* For information on Reference Titre, obtained by calibration with the 1st World Health Organization (WHO) International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR refer to the documentation that came attached to each lot.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Hazard Classification
P210 b3a2 10^{-1} TUBE WITH RED INSERT	400 ng / µL RNA solution ~10% RNA from t(9;22) cell line ~90% RNA from normal cell line	**1 x 18 µL**	-
P210 b3a2 10^{-2} TUBE WITH BLUE INSERT	400 ng / µL RNA solution ~1% RNA from t(9;22) cell line ~99% RNA from normal cell line	**1 x 18 µL**	-
P210 b3a2 10^{-3} TUBE WITH GREEN INSERT	400 ng / µL RNA solution ~0.1% RNA from t(9;22) cell line ~99.9% RNA from normal cell line	**1 x 18 µL**	-
P210 b3a2 10^{-4} TUBE WITH YELLOW INSERT	400 ng / µL RNA solution 0.01% RNA from t(9;22) cell line ~99.99% RNA from normal cell line	**1 x 18 µL**	-
P210 b3a2 $10^{-4.5}$ TUBE WITH VIOLET INSERT	400 ng / µL RNA solution 0.0032% RNA from t(9;22) cell line ~99.997% RNA from normal cell line	**1 x 18 µL**	

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitril powder-free gloves or similar material.
- Vortex mixer.
- 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, 200-1000 µL).
- 1.5 mL polypropylene microtubes for molecular biology
- Molecular biology grade water.
- Reagents for the reverse transcription of the RNA.
- Nucleic acids amplification assays for the amplification and the detection of the cDNA of BCR-ABL rearrangement, t(9;22) translocation, P210 variant, and its quantification normalized in comparison to the cDNA of the ABL gene.
- Programmable heater.

WARNINGS AND PRECAUTIONS

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 potentially infective. 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 / 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 product before running the assay.

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

Do not use the product after the expiry date.

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

Do not use reagents from different batches.

Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified and trained personnel 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.

In case of a manual set-up, 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.

In case of a manual set-up, it is necessary to have lab coats, gloves and tools which are exclusively used 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 used for this type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used 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 used 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 used 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 used exclusively for this specific purpose.

Warnings and precautions specific to components

The tubes containing the mixtures of total RNA **P210 b3a2** must be frozen and thawed for no more than **four times**. Further cycles of freezing and thawing may cause a RNA degradation.

PROCEDURE

The mixtures of total RNA **P210 b3a2** provided by **«PHILADELPHIA P210 RNA Reference»** product are ready to use, so they can be directly used in the required quantity in the reverse transcription reaction.

The cDNA product of the reverse transcription reaction of total RNA **P210 b3a2** can be used in nucleic acids amplification assays.

We suggest to perform the reverse transcription of each of the five mixtures of total RNA **P210 b3a2** in duplicate.

Note: For users of ELITechGroup S.p.A. **«RT - kit plus»** (code BRK200) product: dilute 2,5 µL of each mixture of RNA **P210 b3a2** (equal to 1 µg) with 7.5 µL of **Ultrapure water** (not supplied in the kit), then transfer 10 µL of dilution to the **«monotest»** tubes dedicated to the reverse transcription reactions.

Note: For users of ELITechGroup S.p.A. **«BCR-ABL P210 ELITe MGB® Kit»** (code RTSG07PLD210) product: dilute 0.75 µL of each mixture of RNA **P210 b3a2** (equal to 300 ng) with 9.25 µL of **Ultrapure water** (not supplied in the kit), then transfer 10 µL of dilution to the wells of the **Amplification Microplate** with the complete reaction mixtures.

Note: The **RNA Reference** must be frozen and thawed for no more than **four times**.

PHILADELPHIA P210 RNA Reference
total RNA control for quantitative assay

REF **SPG07-210**

REFERENCES

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M. Baccarani et al. (2013) Blood 122: 872 - 884

S. Branford et al. (2006) Leukemia 20: 1925 - 1930

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MC Muller et al. (2009) Leukemia 23: 1957 - 1963

N. Cross et al. (2009) Best Pract Res Clin Haematol. (2009) 22(3): 355 - 65

H.E. White et al. (2010) Blood: Nov 25;116(22):e111-7

SYMBOLS

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