JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements

CERTIFICATE OF ANALYSIS

ERM®-AD623

SET OF PLASMID SOLUTIONS		
	Number of specific DNA fragments per plasmid	
	Certified value [b]	Uncertainty [c]
BCR-ABL b3a2 transcript [a]	1	negligible
BCR transcript [a]	1	negligible
GUSB transcript [a]	1	negligible

a) The sequence identity has been confirmed by dideoxy termination sequencing of the entire plasmid. The estimated error probability of the sequence identification of each fragment is lower than 0.0002 %.

b) The certified value is traceable to the International System of units (SI).

c) The uncertainty of the certified value refers to a standard uncertainty. It is estimated by a type B evaluation based on information provided in section 6 of the corresponding certification report.

	Copy number concentration of the plasmid [d]	
	Certified value [cp/µL]	Uncertainty [g] [cp/µL]
ERM®-AD623a	1.08×10^{6} [e]	0.13×10^{6}
ERM®-AD623b	1.08×10^{5} [f]	0.11×10^{5}
ERM®-AD623c	1.03×10^{4} [f]	0.10×10^{4}
ERM®-AD623d	1.02×10^{3} [f]	0.09×10^{3}
ERM®-AD623e	1.04×10^{2} [f]	0.10×10^{2}
ERM®-AD623f	10.0 [e]	1.5

d) The copy number concentration of the plasmid has been determined by digital PCR using experimental conditions as described in Annex A of the certification report. This value refers to the copy number concentration of the double stranded plasmid in the solution.

e) Unweighted mean value of 17 independent measurements obtained in three different laboratories. The certified value and its uncertainty are traceable to the International System of units (SI).

f) Unweighted mean value of 18 independent measurements obtained in three different laboratories. The certified value and its uncertainty are traceable to the International System of units (SI).

g) The certified uncertainty is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of approximately 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2009.

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 2 µL.

Accepted as an ERM®, Geel, September 2012

Latest revision: February 2015

Signed:

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DESCRIPTION OF THE MATERIAL

ERM-AD623 is a set of six solutions of double stranded plasmid DNA (ERM-AD623a, ERM-AD623b, ERM-AD623c, ERM-AD623d, ERM-AD623e and ERM-AD623f). The plasmid contains a specific DNA fragment from the fusion transcript of the break cluster region gene (*BCR)* and the c-abl oncogene1 (*ABL*), called *BCR-ABL* b3a2 transcript. Additionally the plasmid also contains specific DNA fragments from the *BCR* transcript and the glucuronidase beta gene (*GUSB*) transcript. This plasmid was diluted to six different concentration levels. Solutions are made of 1 mmol/L Tris, 0.01 mmol/L EDTA pH 8.0 and supplemented with 50 ng/µL of transfer RNA from *Escherichia coli*. Each of the six vials contains approximately 600 µL of plasmid solution. Following the release of ERM-AD623, an additional study has been carried out which allowed to lower the minimum sample intake from 5 µL to 2 µL.

ANALYTICAL METHODS USED FOR CERTIFICATION

Dideoxy terminator sequencing to determine the sequence identity of the plasmid
Digital PCR to determine the copy number concentration of the plasmid.

PARTICIPANTS

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SAFETY INFORMATION

The usual laboratory safety precautions apply.

INSTRUCTIONS FOR USE AND INTENDED USE

ERM-AD623 is intended for the calibration of quantitative real time PCR (qPCR) methods measuring the number of *BCR-ABL* b3a2 transcripts in relation to the number of transcripts of one of the following control genes: *BCR*, *ABL* or *GUSB*. The general PCR conditions listed in the Tables A1 and A2 of the certification report were used to obtain the certified copy number concentrations of ERM-AD623. A limited commutability study has shown that ERM-AD623 can calibrate different qPCR methods listed in Annex H of the certification report. Suitability of ERM-AD623 for the quantification of the *BCR-ABL* b2a2 transcript would have to be verified. ERM-AD623 can be used directly in the routine analysis of cDNA samples from patients or indirectly for calibrating in-house plasmid standards.
This set contains six concentrations which should be used to construct the calibration curves for both the *BCR-ABL* b3a2 transcript and the control gene of choice. The certified values on the copy number concentrations refer to copy numbers of the double stranded plasmid and these values shall be multiplied by 2 when measuring single stranded cDNA samples from patients. Two values are important to report for the cDNA samples from patients: the copy number ratio *BCR-ABL* fragment/control gene fragment and the copy number concentration of the control gene fragment. As the sequences of the *BCR-ABL* b3a2 transcript and the control gene transcript are located on the same plasmid, the contribution of ERM-AD623 to the uncertainty associated with the measured value for the copy number ratio *BCR-ABL* fragment/control gene fragment is negligible. When reporting the copy number concentration of the control gene fragment, the uncertainty associated with the certified copy number concentrations of the ERM-AD623 plasmid solutions must be taken in account.
To reduce the risk of contamination, opening of the vials and dispensing of the solutions should be done in a laminar flow cabinet. Special care should be taken to avoid aerosol formation when performing the experiments.

STORAGE

The materials shall be stored at -20 °C ± 5 °C.

Under the condition that contaminations have been excluded, the solutions can be used for several experiments. The material should however not pass more than 10 freeze/thaw cycles. The material can also be stored at 4°C ± 3 °C for up to 4 weeks as it was verified that changes to the certified concentration observed during that period are not significant. It is advisable to close the vial with the original screw cap after use.

The plasmid containing solution should not be exposed to direct sun light.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

LEGAL NOTICE

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NOTE

European Reference Materials ERM-AD623a, ERM-AD623b, ERM-AD623c, ERM-AD623d, ERM-AD623e and ERM-AD623f were produced and certified under the responsibility of the Institute for Reference Materials and Measurements of the European Commission's Joint Research Centre according to the principles laid down in the technical guidelines of the European Reference Materials® co-operation agreement between BAM-IRMM-LGC. Information on these guidelines is available on the internet (http://www.erm-crm.org). A detailed technical report is also available on www.erm-crm.org. A paper copy can be obtained from the Joint Research Centre, Institute for Reference Materials and Measurements on request.

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