POSITIVE CONTROL pBALF5 (EBV)

CATALOG NUMBER: DPC0005 **LOT NUMBER:** #

RECOMBINANT PLASMID: EBV BALF5 Positive Control DNA (Yoshinori *et al.*, 2010).

DESCRIPTION: plasmid dsDNA containing the complete sequence of the gene BALF5 amplified from the complete genome isolated from human Epstein-Barr virus.

QUANTITY: 100 µl at 2.00 ng/ml (30,147,861.16 DNA copies, 301,478.61 copies/µl)

BLAST ANALYSIS: KC207813.1 complement (152783..155830)

PRESENTATION: liquid DNA solution

SOURCE: recombinant DNA propagated and purified from *Escherichia coli*

MOLECULAR WEIGHT: 4 megadalton, 6054 base pairs

BATCH COMPOSITION:

COMPONENTS	COMPOSITION
pBALF5	recombinant plasmid containing the complete ORF of EBV BALF5 gene
Storage buffer	DNase-free sterile TE 1x

QUALITY CONTROL:

1. INITIAL DNA CONCENTRATION DETERMINED ESPECTROPHOTOMETRICALLY

DO_{260} = 0.281 (dil 1:200)
CONCENTRATION INITIAL STOCK*: 2.80 mg/ml

* Direct measurements of nucleic acid samples at OD_{260} can be converted to concentration using the Beer-Lambert law which relates absorbance to concentration using the pathlength of the measurement and an extinction coefficient of the DNA (1/50 µg/ml*cm) (Maniatis *et al.*, 1982).

PURITY: A_{260}/A_{280}=1.82

2. DETERMINATION OF DNA CONCENTRATION BY ESPECTROFLUORIMETER

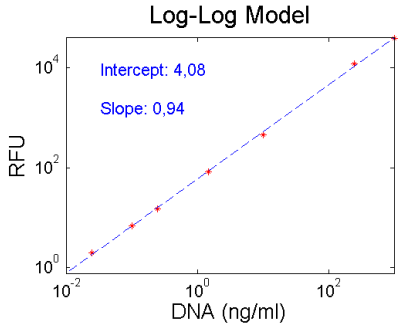

Figure 1. Calibration curve of lambda DNA from 25 pg/ml to 1000 ng/ml, using the PicoGreen dsDNA quantification reagent.

RFU: 114

CONCENTRATION: approx. 2 ng/ml

DATE OF BATCH DILUTION: 12/02/2013

3. PURITY CONTROL IN AGAROSE 0.8%

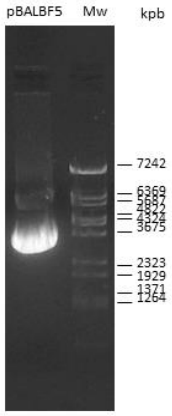

Figure 2. Electrophoresis analysis (0.8%) of 1 µl of initial isolated plasmid pBALF5. Content of closed-circular form > 95%.

4. PCR CONTROL IN AGAROSE:

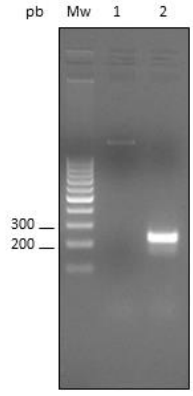

Figure 3. Electrophoresis analysis (2%) of PCR assay on pBALF5 DNA, with internal oligonucleotides. The amplified fragment has a molecular weight of 228 pb. Well 1, negative control; well 2, 1 µl of pBALF5 2 ng/ml.

* Internal oligonucleotide sequences are: 5´-GGAAGCCCTCTGGAC TTC-3´and 5´-GCAAACTCCACGTCCAGAC-3. Suggested concentration in a PCR assay, 0.5 µM

5. qPCR ASSAY:

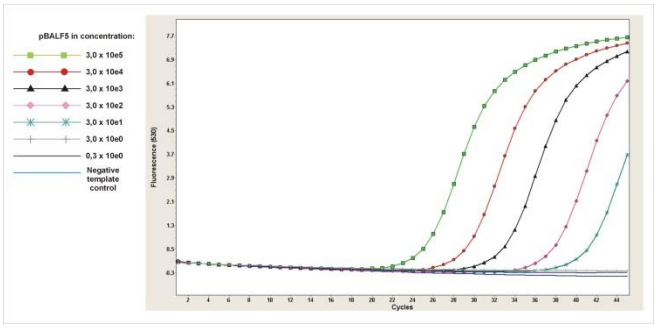

Figure 4. qPCR assay using pBALF5 as positive control. Data provided by M. Przybylski and T. Dzieciatkowski, Medical University of Warsaw.

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6. ABSENCE OF NUCLEASES: absence of contaminating RNA and genomic DNA impurities.

LOT SPECIFICATIONS:

1. CONCENTRATION: 2 ng/ml

2. TOTAL QUANTITY PER ALIQUOT: 200 pg (30,147,861.16 DNA copies)

3. TOTAL VOLUME PER ALIQUOT: 0.1 ml

4. SUGGESTED TITER BY PCR: 2 µl of a 1:100 for traditional PCR, which corresponds to 40 fg (6029.56 copies).

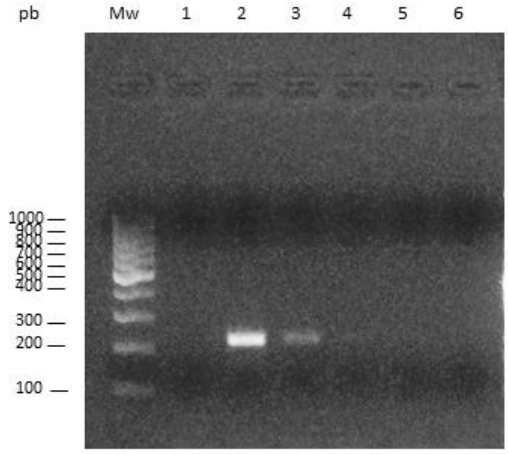

Figure 5. Electrophoresis analysis (2%) of PCR assay on pBALF5 DNA, with internal oligonucleotides. Well numbers correspond to:

1-Negative control
2-2 µl of the non-diluted solution
3-2 µl of a 1/10 dilution
4-2 µl of a 1/100 dilution (last dil. detected in an agarose gel)
5-2 µl of a 1/1000 dilution
6-2 µl of a 1/10,000 dilution

5. STORAGE: DNA is shipped with dry ice. Upon arrival they should be aliquoted and stored at -20ºC to -80ºC. Avoid multiple freeze/thaw cycles

6. APPLICATIONS: for PCR and qPCR assays. Where this product has not been tested for use in a particular technique, this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates.

7. OBSERVATIONS: DNA in so high diluted solutions (< 1000 copies/µl) is very unstable, therefore we recommend that these kind of dilutions should not be stored.

RELATED PRODUCTS:

None.

BIBLIOGRAPHY:

Yoshinori Ito, Shunji Takakura, Satoshi Ichiyama, Mitsuharu Ueda, Yukio Ando, Kazuyuki Matsuda, Eiko Hidaka, Kaname Nakatani, Junji Nishioka, Tsutomu Nobori, Naoki Kajiyama and Hiroshi Kimura. Multicenter evaluation of prototype real-time PCR assays for Epstein-Barr virus and cytomegalovirus DNA in whole blood samples from transplant recipients. *Microbiol Immunol* 2010; 54: 516–522.

Maniatis T, Fritsch EF and Sambrook J. Molecular cloning a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor NY (1982).

Important Notes: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µl or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the containers cap.

Although plasmids are isolated from non-pathogenic *E. coli,* and bacterial integrity is destroyed during purification, the plasmid preparation should be handled as potentially infectious.

NOT FOR DIAGNOSTIC USE, FOR RESEARCH USE ONLY

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