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CERTIFICATE OF QUALITY

Catalog number:

- □ P50-P
- □ P100-P
- □ P300-P
- □ P500-P

Pf1 magnetic resonance co-solvent

Protease free (Pf1 bacteriophage strain LP11-92)

The bacteriophage Pf1 strain LP11-92 was propagated in *Pseudomonas aeruginosa* strain LA23-99; it was purified by ultracentrifugation and ultrafiltration. The homogeneity of the phage obtained is more than 98 % according to the electroforetic analysis of phage DNA on agarose gel stained by ethidium bromide as shown in Fig.1, Appendix 1. Concentration of phage was determined by UV absorbance at 270 nm, with the extinction coefficient 2.25 cm mg^{-1} ml^{-1}. The purified phage was kept at 0^{o}C. Protease contamination was tested as shown in Fig. 2 of Appendix 1.

Storage buffer: 10 mM potassium phosphate buffer pH 7.6 containing 2 mM $MgCl_2$ and 0.05% NaN_3, otherwise indicated below:

……

……

Date of expiration: .....if kept at 0^{o} to 5^{o}C. Do not freeze!

Lot No: .....

Concentration: ..... mg/ml

Protease contamination: .....

Issued (date): ……. by Dr. Andris Zeltins
R&D Manager, ASLA BIOTECH Ltd.

This product can be used for research purposes only!
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CERTIFICATE OF QUALITY

Catalog number:

- □ P50-RNA
- □ P100-RNA
- □ P300-RNA
- □ P500-RNA

Pf1 magnetic resonance co-solvent

RNAse and protease free (Pf1 bacteriophage strain LP11-92)

The bacteriophage Pf1 strain LP11-92 was propagated in *Pseudomonas aeruginosa* strain LA23-99; it was purified by ultracentrifugation and ultrafiltration. The homogeneity of the phage obtained is more than 98 % according to the electroforetic analysis of phage DNA on agarose gel stained by ethidium bromide (as in Fig.1, appendix 1). Concentration of phage was determined by UV absorbance at 270 nm, with the extinction coefficient 2.25 cm $mg^{-1}ml^{-1}$. The purified phage was kept at $0^{o}C$. Protease and RNAse contaminations was tested as shown in Fig. 2, and Fig.3 of Appendix 1, respectively.

Storage buffer: **10 mM potassium phosphate buffer pH 7.6 in DEPC water, otherwise indicated below:**

……

……

Date of expiration: ..... **if kept at 0^{o} to $5^{o}C$. Do not freeze!**

Lot No: .....

Concentration: ..... **mg/ml**

Protease contamination: .....

RNAse contamination: .....

Issued (date): ..... **by Dr. Andris Zeltins**
R&D Manager, ASLA BIOTECH Ltd.

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APPENDIX 1: Quality tests

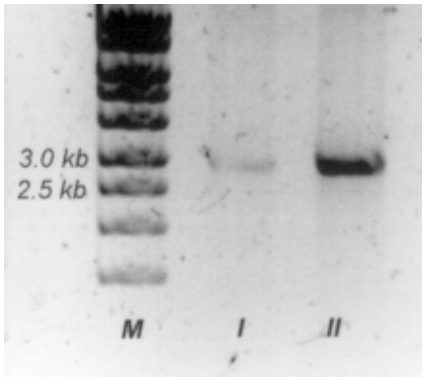

Figure 1. **Phage homogeneity.**
Pf1 DNA after phenol/chloroform treatment and isopropanol precipitation:
I – an aliquot corresponding to 1.5 μg Pf1
II – an aliquot corresponding to 7.5 μg Pf1
The electroforetic analysis of phage DNA on 0.8% agarose gel in Tris-borate buffer stained by ethidium bromide.

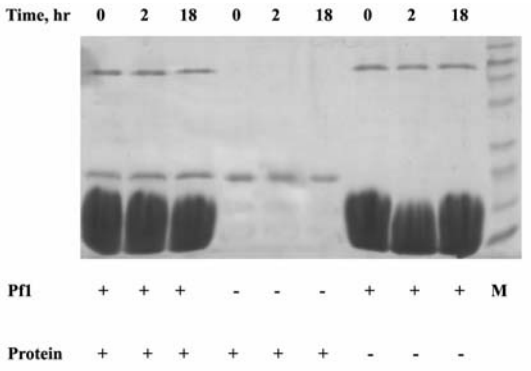

Figure 2. **Presence of proteases.**
Protease contamination test by incubation of 2.5 mg/ml reference protein emilin in the presence of the 25 mg/ml Pf1, followed by 15% SDS-PAAGE during the times indicated. The protein quantity is measured by the densitometry of reference protein band on scanned gel image by GelD software (ASLA BIOTECH). The protease contamination is non-essential for the examined lot of Pf1 MRC (numbers are not shown).

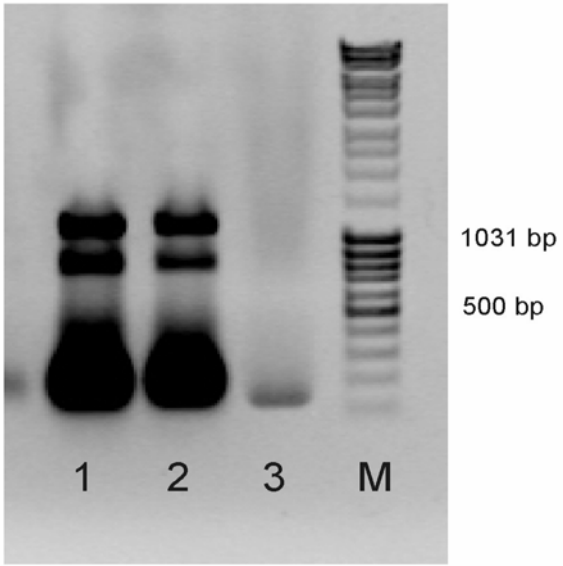

Figure 3. **Presence of RNAses.**
E.coli total RNA was isolated using phenol containing Tri-Reagent (Sigma) according to manufacturer's recommendations. 12 μg of RNA were incubated with 25 mg/ml Pf1 MRC for 120 min at room temperature and 48 hr at + 4°C. After incubation, total RNA was separated by repeated Tri-reagent treatment. RNA was analyzed by 1.5% agarose gel electrophoresis.

1. Pf1 isolated according to the RNAse free protocol and incubated with RNA as indicated above.
2. Pf1 isolated according to RNAse free protocol, mixed with RNA and frozen immediately at –20°C.
3. Pf1 isolated according to RNAse free protocol, mixed with RNA and incubated with RNA plus 1.0 ug RNAse A.

M Molecular size markers

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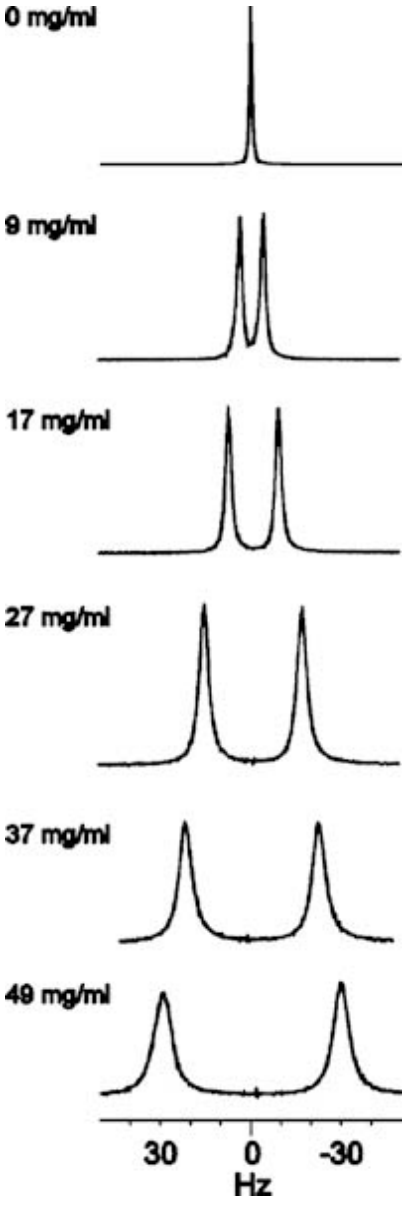
APPENDIX 2: Splitting of Spectra

Figure 1. Quadrupolar splittings of the ^2H NMR signal of D_2O in the presence of different concentrations of Pf1 phage LP11-92, recorded at 25°C.



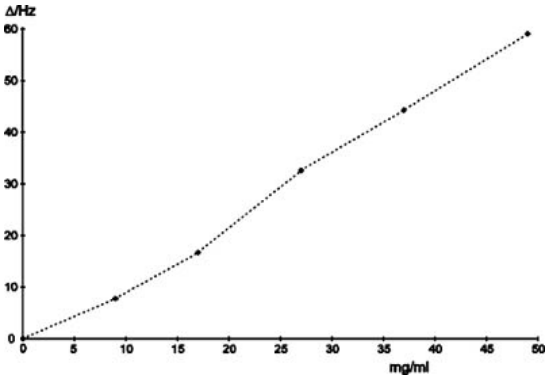


Figure 2. The relationship between LP11-92 phage concentration and quadrupolar splittings of the ^2H NMR signal of D_2O (Δ/Hz).

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APPENDIX 3: Technical tips

Pf1 MR co-solvent: Some useful advises

1. **Attention**: the phage suspension is quite **viscous** because of the relatively long particles and it can bring some problems when you fill in the tube for NMR sample and mix your sample with the phage. We suggest to use very simple adapter which you can make by yourself (shown in the figure below). Just attach the Teflon tube 1.5 to 3 mm in diameter to the 1 ml blue tip for the automatic pipette with the 1 cm long peace of silicon tubing of slightly smaller diameter. The adapter is disposable, so you can be sure about the purity and accuracy of your manipulations. The phage suspension should be mixed with your protein or nucleic acid of interest by intensive pipetting followed by spinning at 10,000 rpm on the table centrifuge to remove the burbles.

2. **To change the buffer** just spin down the phage suspension at 95,000 rpm in a Beckman TLA-100.3 rotor for 1 hr at 5^{o}C (alternatively at 40,000 in a Vti50 rotor for 6 hr) and resuspend the phage in the buffer of your choice. **Attention**: the recovery may be 50 to 70%.
3. **Do not freeze the phage.** Keep it at +4^{o}C. The phage worked properly in deuterium spectra splitting after 6 month of incubation at +5^{o}C.

References

1. Hansen MR, Mueller L, Pardi A. (1998) Tunable alignment of macromolecules by filamentous phage yields dipolar coupling interactions. *Nat Struct Biol* Dec;5(12):1065-74
2. Hansen MR, Hanson P, Pardi A. (2000) Filamentous bacteriophage for aligning RNA, DNA, and proteins for measurement of nuclear magnetic resonance dipolar coupling interactions. *Methods Enzymol*; 317:220-40
3. Otting G. 2000 *(Pers.com.)*