

Basic Principles for the Best Results With pFix5 Standard

Content

- 1 x vial of 1 mL sample (250 µg of plasmid pFix5) (Cat. No. BIA-PFIX5.1.1)
Packing buffer: 10 mM TRIS, 1 mM EDTA, pH 8.0

Equipment Required

- PATfix® -model-pDNA (analytical system with quaternary pump) (Cat. No. PAT0029)

Consumables That Are Not Included

- CIMac™ pDNA column (1.4 µm) (Cat. No. 150.8501-1.4), vials for the PATfix® system
- 0.22 µm PES filter

Introduction

Plasmids are small, circular DNA molecules, physically separated from chromosomal DNA and can be replicated independently within a cell. They naturally exist in bacteria cells and are sometimes present in archaea and eukaryotic organisms. Often plasmids contain additional genes, arming plasmids with genetic advantages, such as antibiotic resistance. Plasmids have a wide range of lengths, from thousand base pairs to few thousand base pairs. Naturally they are involved in reproduction processes, resistance against antibiotics or poisons, producing bacteriocins and can digest unusual substances. Due to many possibilities for application in biotechnology, their use in pharmaceutical field has grown significantly over last decades. Genetically constructed plasmids, named recombinant plasmids, are widely used as vectors in genetic engineering. Plasmid DNA is found in several conformations, such as open circular (OC) and supercoiled (SC) isoforms. In production of plasmid DNA for gene therapy and DNA vaccination it is important to distinguish between those two isoforms.

Plasmid pFix5 standard is plasmid DNA of 4.7 kb length. It is isolated from *E. coli* microorganism and chromatographically purified. Plasmid DNA standard is used for System Suitability Testing and for preparation of the standard curve. The standard contains OC and SC plasmid DNA isoforms.

The product is intended for research use only. Material has not been retrieved from humans and it is not of animal origin. The biological material does not produce antigens or contain genes of livestock or avian disease agents and does not produce monoclonal antibodies directed against livestock or avian disease agents and does not express antigens of livestock or poultry disease agents. The material does not contain any animal derived additives.

Standard Storage

Long term storage: -80 °C

Short term storage: pFix5 can be stored up to 6 months at -20 °C.

Standard shipping up to 8 °C is recommended.

Avoid multiple freeze-thaw cycles.

Recommended Procedure

This procedure describes the experimental materials and methods used to perform analysis for the standard curve.

Standard preparation: pDNA standard stock solution (pFix5) with concentration 250 µg/mL is your standard stock solution. For System Suitability Testing load approximately 0.5 µg of pDNA with injection volume of 50 µL. Dilute with dilution buffer.

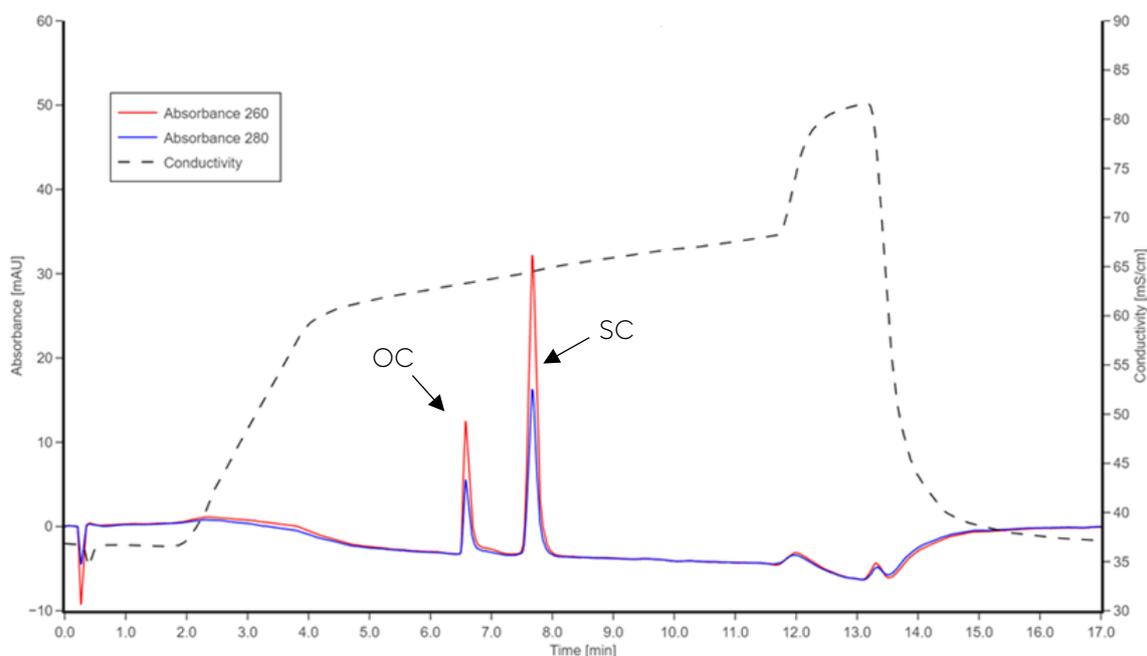
For the calibration curve, prepare desired concentration of pDNA working solution by pipetting standard stock solution (pFix5) and adding buffer for dilution. Prepare at least 6 calibration standards of different concentration in range 1 µg/mL to 25 µg/mL by taking a corresponding volume of pDNA working solution and dilution buffer.

Buffers and solvents:

Composition of dilution buffer: 100 mM TRIS, 300 mM Guanidine-HCl, pH 8.00

Representative Results Using pFix5 Standard

Figure 1: pDNA analytics of pFix5 plasmid standard (10 µg/mL) on 1.4 µm pDNA column. Monitored absorbance at 260 and 280 nm and conductivity. OC – open-circular plasmid DNA; SC – supercoiled plasmid DNA.



Ordering Information

Cat No.	Product Name
pDNA applications	
BIA-PFIX5.1.1	pDNA standard pFix5 sc/oc (250 µg)
BIA-PFIX15.1.1	pDNA standard pFix15 sc/oc (250 µg)
150.8501-1.4	CIMac™ pDNA 0.3 mL Analytical Column (1.4 µm channels)
150.8501-6	CIMac™ pDNA 0.3 mL Analytical Column (6 µm channels)
PAT0029	PATfix® - model-pDNA (analytical system with quaternary pump)
mRNA applications	
BIA-MFIX4.1.1	mRNA standard mFix4 (150 µg)
110.1219-2	CIMac™ Oligo dT18 0.1 mL Analytical Column (C12 Linker) (2 µm channels)
110.5118-2	CIMac PrimaS™ 0.1 mL Analytical Column (2 µm channels)
BIA-110.9001-2	CIMac™ SDVB 0.1 mL Analytical Column (2 µm channels)
PAT0050	PATfix® - model-mRNA (analytical system with quaternary pump)

Sartorius BIA Separations
Mirce 21
5270 Ajdovscina, Slovenia

Phone: +386 59 699 500
www.biaseparations.com

The information and figures contained in these instructions correspond to the version date specified below.

Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.

Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

Copyright notice:

These instructions, including all components, are protected by copyright.

Any use beyond the limits of the copyright law is not permitted without our approval.

This applies in particular to reprinting, translation and editing irrespective of the type of media used.

Last updated:
09 | 2024