

Basic Principles for the Best Results With mFix4 mRNA Standard

Content

- 1 x vial of 300 µL sample (150 µg of mRNA standard) (Cat. No. BIA-MFIX4.1.1)
Packing buffer: 1 mM Sodium Citrate, pH 6.4

Equipment Required

- PATfix®-model-mRNA (analytical system with quaternary pump) (Cat. No. PAT0050)

Consumables That Are Not Included

- CIMac™ Oligo dT column (Cat. No. 110.1219-2), CIMac PrimaS™ column (Cat. No. 110.5118-2) or CIMac™ SDVB column (Cat. No. BIA-110.9001-2), vials for the PATfix® system
- 0.22 µm PES filter

Introduction

Messenger RNA (mRNA) is a type of RNA molecule carrying the coding sequences for protein synthesis. It is naturally produced during the process of transcription from a DNA template in cells. mRNA can also be retrieved artificially, e. g. by IVT reaction, and used as vector for mRNA vaccines and treatments. All major components of the IVT reaction (nucleotides, capping reagent, enzymes, and DNA template) may be present in any mRNA sample. In addition, impurities such as shorter or incomplete RNA fragments and dsRNA may be present. Contaminants may also come from the mRNA in vitro instability, caused by spontaneous hydrolyzation of the mRNA backbone. There is a constant threat of RNase contamination that can quickly degrade mRNA molecules. These issues can be mitigated using appropriate analytical tools throughout the mRNA production and purification steps.

The mFix4 mRNA standard is a 3969 nt long mRNA molecule with a polyA tail. Molecule is produced by IVT reaction with DNA template retrieved from recombinant microbial expression (*E. coli*). Standard is used for System Suitability Testing. The standard contains polyadenylated mRNA.

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 contains 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: mFix4 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

mRNA standard can be used for three complementary analytics: Oligo dT (affinity chromatography with CIMac™ Oligo dT column), PrimaS (bimodal anion exchange and hydrogen bonding with CIMac PrimaS™ column) and SDVB (reverse phase with CIMac™ SDVB column). This procedure describes the experimental materials and methods to prepare standard working solution for each analytics.

Oligo dT Analytics

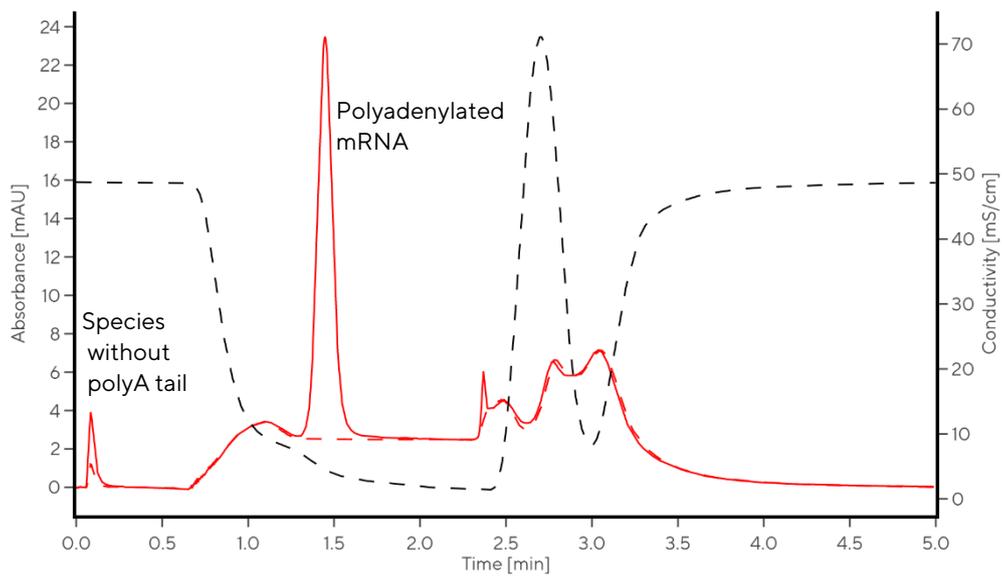
Standard preparation: mRNA standard solution (mFix4) with concentration 500 µg/mL is your standard solution. Load approximately 0.25 µg of mRNA with injection volume of 50 µL. Dilute with buffer for dilution.

Buffers and solvents:

Composition of dilution buffer: 30 mM HEPES, 30 mM Na phosphate, 0.5 M NaCl pH 7.4

Representative Chromatograms of mFix4 mRNA Standard

Figure 1: mRNA Oligo dT analytics of mFix4 mRNA standard (UV 260 nm)



PrimaS Analytics

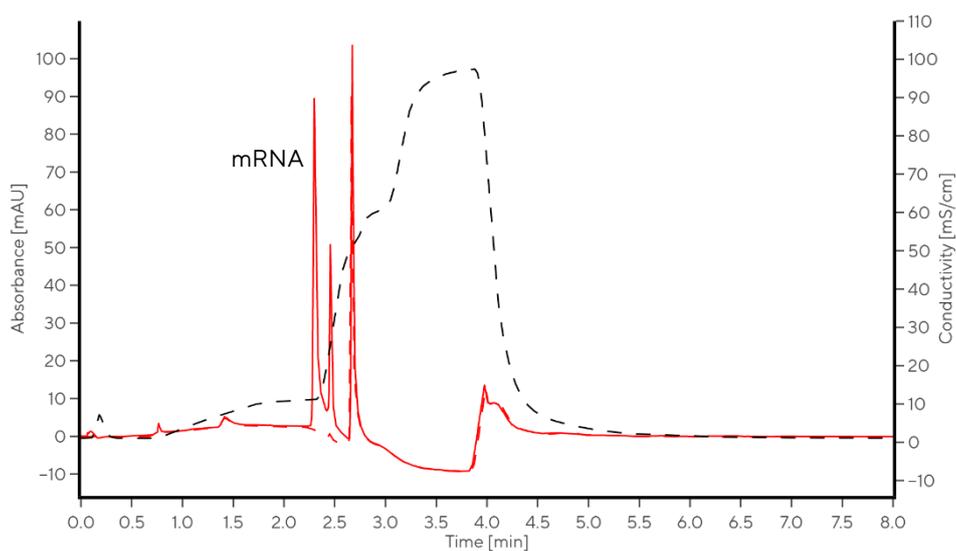
Standard preparation: mRNA standard solution (mFix4) with concentration 500 µg/mL is your standard solution. Load approximately 0.5 µg of mRNA with injection volume of 100 µL. Dilute with buffer for dilution.

Buffers and solvents:

Composition of dilution buffer: 50 mM HEPES, pH 7.0

Representative Chromatograms of mFix4 mRNA Standard

Figure 2: mRNA PrimaS analytics of mFix4 mRNA standard (UV 260 nm)



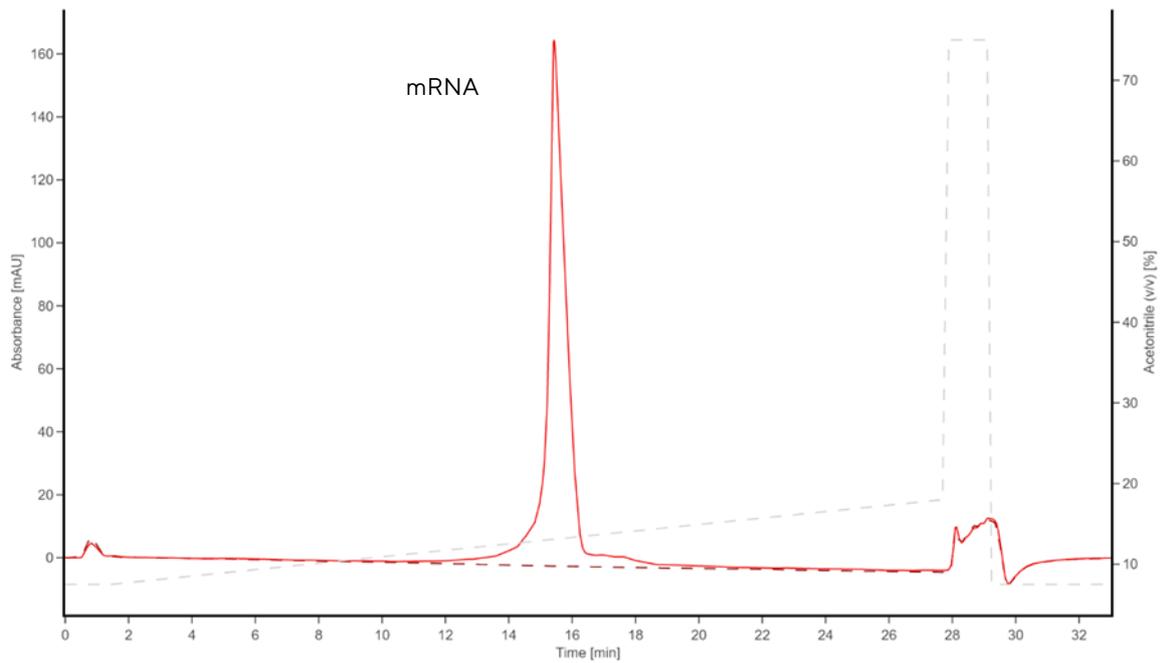
SDVB Analytics

Standard preparation: mRNA standard solution (mFix4) with concentration 500 µg/mL is your standard solution. Load approximately 0.5 µg of mRNA with injection volume of 250 µL. Dilute with buffer for dilution.

Buffers and solvents: Prepare buffer for dilution.
Composition of dilution buffer: 50 mM TEAA, 7.5% m/m ACN, pH 7.0

Representative Chromatograms of mFix4 mRNA Standard

Figure 3: mRNA SDVB analytics of mFix4 mRNA standard (UV 260 nm)



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)

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