

ExpressArt® FFPE RNA Enhance

Compounds for improved nucleic acid recovery from FFPE samples

Catalogue No. 8990-M50

sufficient for 50 ml lysate (100 samples)

suitable for RNA isolation with spin column technology

Application for the challenging situation

- **FFPE (formalin-fixed, paraffin-embedded) samples**

Content

DeCrossLinker (DCL) is provided as 100x stock solution for 50 ml lysate or 100 standard RNA isolations	500 µl
NucleoGuard (NG) is provided as 100x stock solution for 50 ml lysate or 100 standard RNA isolations.	500 µl

Storage

Store at -20°C ; NucleoGuard can be stored at room temperature.

DeCrossLinker (DCL) and NucleoGuard (NG) as additives in RNA isolation protocols

DeCrossLinker is a small chemical compound with the ability to (partially) revert cross-links in nucleic acids during their isolation from FFPE samples.

NucleoGuard is a small chemical compound that forms aggregates which act as a mimic for nucleic acids (RNA and DNA) and its application in rather high concentrations results in very effective, competitive inhibition of nucleic acid recognizing enzymes, including RNases, DNases, nucleases as well as nucleic acid polymerases.

These properties ensure

- good penetration of biological samples
- full activity in presence of denaturing agents, like GTC and SDS
- full activity in presence of proteinase K

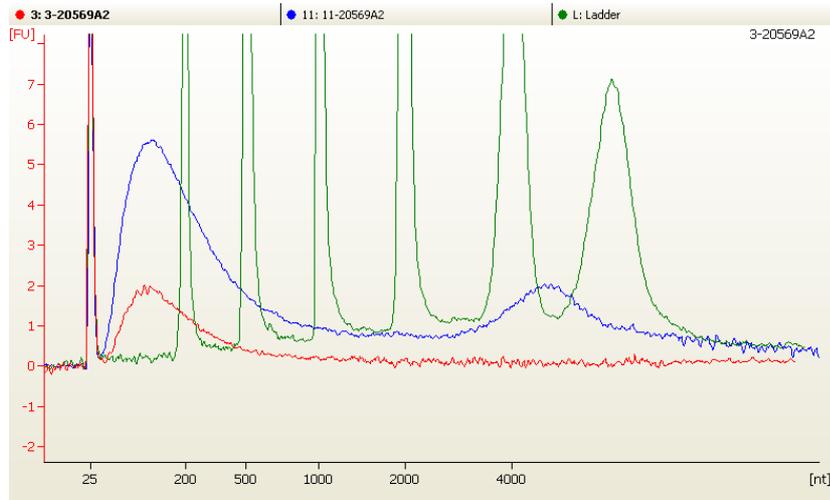
- **Note: DeCrossLinker is an inhibitor of polymerases. Its reliable and quantitative removal is achieved by standard spin column purification kits, like RNeasy (Qiagen), HighPure (Roche) etc.**

The combination of DeCrossLinker and NucleoGuard is especially recommended

- for RNA isolation from **FFPE (formalin-fixed, paraffin-embedded) samples**.

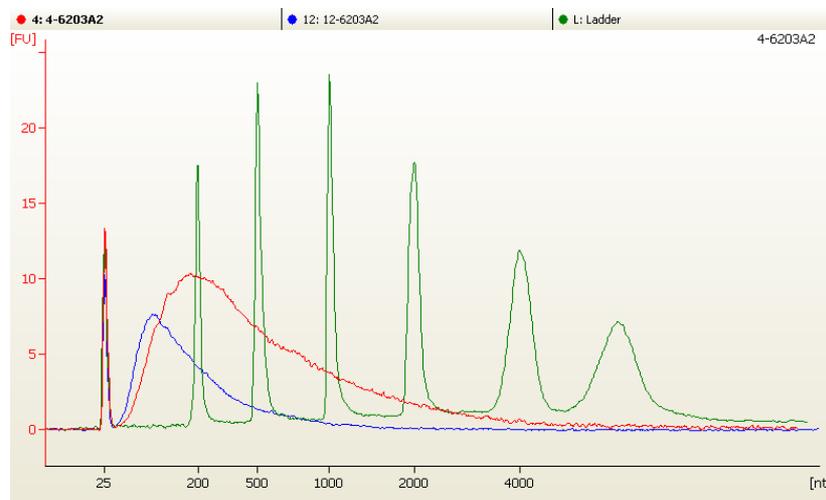
Both reagents together help in preserving the size of FFPE-RNAs, and in resolution of high molecular weight aggregates that can interfere with the activity of RNA (and DNA) templates.

Improved Quality of FFPE-RNAs with ExpressArt additives



Resolution of high molecular weight aggregates

RNA profiles of parallel aliquots. RNA isolation with standard commercial FFPE kit (similar results with kits from Qiagen or Roche) was performed without additives (**blue**), and with ExpressArt DCL & NG additives (**red**). The evident resolution of aggregates (at ≥ 4000 nt) resulted in improved template performance, as illustrated in RT-qPCR data with reduced cycle numbers, ΔCt appr. -3.5.



Recovery of larger RNAs

RNA profiles of parallel aliquots. RNA isolation with a standard commercial FFPE kit was performed without additives (blue), and with ExpressArt DCL & NG additives (red). The size shift to a peak at appr. 200 nt resulted in improved template performance, as illustrated in RT-qPCR data with reduced cycle numbers, ΔCt appr. -3.

Protocol

The reagent DeCrossLinker, in combination with NucleoGuard, can be combined with the RNA isolation method for FFPE samples of your choice:

supplement your standard FFPE lysis reagent (in general, lysis buffer containing a proteinase K) with 1% (v/v) of DeCrossLinker stock solution, and proceed as usual. For further improvement, we strongly recommend to include also 1% (v/v) NucleoGuard stock solution.

Subsequent to spin column elution, we recommend to perform alcohol precipitation for concentration (and for storage or shipping of RNA samples):

To your aqueous RNA solution, add 0.1 volume of 3 M sodium acetate and 1 µl of P-Carrier (**ExpressArt Pico RNA Care, Cat.-No. 8999-A100**). Mix and add ethanol (2.2 volumes). Mix and incubate for 10 min on ice.

If storage or shipping is desired, use this alcoholic suspension of RNA and keep at or below -20°C.

For RNA recovery, spin for 20 min with 14,000g at 4°C. Remove supernatant with a pipette and wash with 80% ethanol. Spin again for 10 min with 14,000g at 4°C. Remove supernatant, spin briefly and carefully remove all liquid. Then air dry for 5 min and dissolve in desired volume of RNase-free water.

The recovered RNA is free of salt and buffer, and is ideal for quality control with capillary electrophoresis systems like Bioanalyzer 2100 (Agilent) or Experion (BioRad). See also Agilent Application Note 5989-0712EN (March, 2004); available on request from krupp@amp-tec.com.

Ideally, use the isolated RNA immediately for mRNA amplification with the appropriate ExpressArt® mRNA amplification kit.



AmpTec GmbH

Koenigstrasse 4a

22767 Hamburg

Germany

Tel: +49 (0)40 636 747 22

Fax:+49 (0)40 636 747 19

Technical Support:

"Dr. Guido Krupp" <krupp@amp-tec.com>

"Dr. Peter Scheinert" <scheinert@amp-tec.com >

www.amp-tec.com