

## Effect of Matrix on UV Determination of mRNA Content

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### Higher Conductivity Results in Lower $A_{260}$ Measurements of Pure mRNA Samples

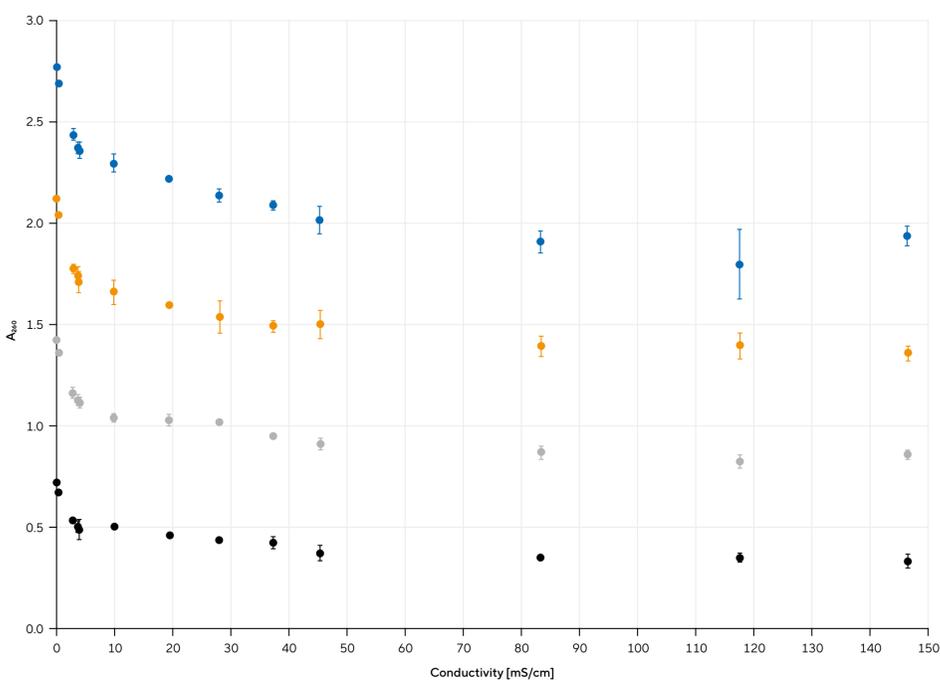
Microvolume spectrophotometers are commonly used as a quick and easy method to measure the concentration and purity of nucleic acids.

The downstream process (DSP) for mRNA purification includes unit operations with salt concentrations up to 2.75 M (for hydrophobic interaction chromatography with CIMmultus® C4 HLD columns) or 1.25 M (for affinity chromatography with CIMmultus® Oligo dT) during load and low salt concentrations during elution (Table 1).

**Table 1:** Typical Matrices Used for CIMmultus® Oligo dT- and C4 HLD-based Purification of mRNA

mRNA Purification Step	Commonly Used Matrices
CIMmultus® Oligo dT column	Load: 50 mM Na-phosphate + 1.25 M NaCl, pH 7.4
	Elution: ddH <sub>2</sub> O
CIMmultus® C4 HLD column	Load: 50 mM Tris + 2.75 M NaCl, pH 7.2
	Elution: 50 mM Tris, pH 7.2
Storage buffer	1 mM Na-citrate, pH 6.0 or ddH <sub>2</sub> O

**Figure 1:**  $A_{260}$  Measurements for mRNA in Matrices With Different Conductivities



Note. mRNA was diluted to nominal concentration 25 ng/µL (black), 50 ng/µL (grey), 75 ng/µL (orange) and 100 ng/µL (blue) in matrices with different conductivities. The average value of triplicate measurements is plotted for each sample, error bars denote standard deviation.

**Table 2:** Formula for Determining of  $A_{260}$  Value With Conductivity Correction

Statistical Analysis		
Factor Name	Estimate	Standard Error
i	-0.0252	0.0496
c	-0.0051	0.0005
m	0.8244	0.0235
D	5.6E-05	1.0E-05

Equation 1:

$$A_{260}(\text{ddH}_2\text{O}) = \frac{A_{260}(\text{solution}) - c \times \kappa - d \times \kappa^2 - i}{m}$$

Note. The formula was derived on the basis of the experimental set to exclude the effect of conductivity on absorbance measurements at 260 nm. The  $A_{260}$  value for mRNA in a matrix and conductivity ( $\kappa$ , in mS/cm) for the matrix, are required for more accurate determination of mRNA content.

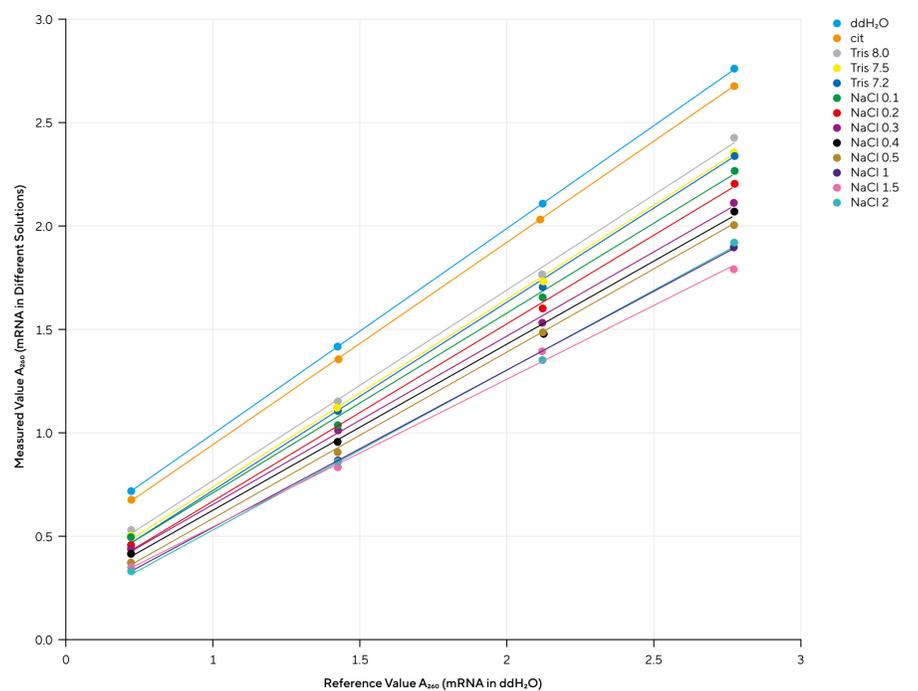
### Deriving a Formula for a More Accurate mRNA Quantification

Spectrophotometric measurement of absorbance at 260 nm ( $A_{260}$ ) is used to calculate mRNA concentration using modified Beer-Lambert Law:  $c = A_{260} \times f$ . Microvolume spectrophotometers use averaged factors  $f = 40 \text{ ng}/\mu\text{L}$  for ssRNA,  $f = 50 \text{ ng}/\mu\text{L}$  for dsDNA, and  $33 \text{ ng}/\mu\text{L}$  for ssDNA.

The effect of matrix conductivity on UV absorbance has been previously reported<sup>1</sup>, but not yet explored for matrices relevant to mRNA purification.  $A_{260}$  variations due to matrix conductivity can lead to errors in mass balance calculations, particularly when load and elution conductivities differ significantly.

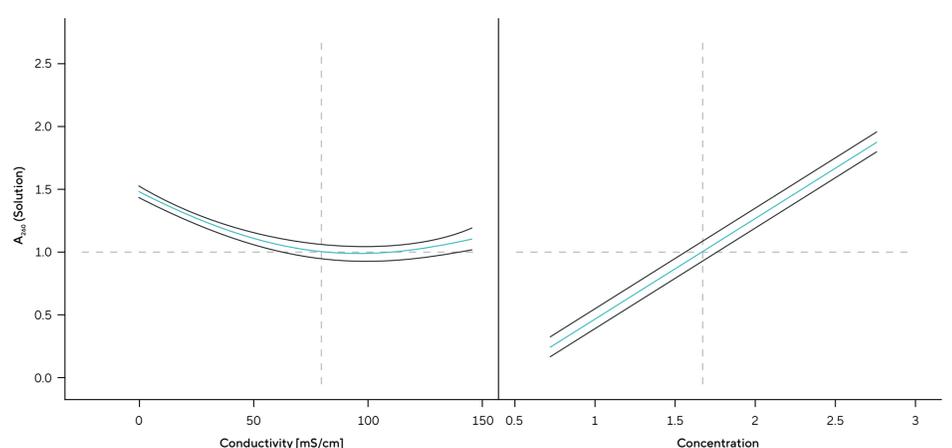
We tested the effect of process-relevant NaCl concentrations on  $A_{260}$  experimentally and derived a mathematical model to correct for the effect of matrix conductivity on  $A_{260}$ .

**Figure 2:** Comparison of  $A_{260}$  Measurements in ddH<sub>2</sub>O And Matrices With Different Conductivities



Note.  $A_{260}$  value for four nominal mRNA concentrations (25–100 ng/mL) were measured against mRNA in ddH<sub>2</sub>O as a reference (x-axis). Measured  $A_{260}$  values for mRNA in tested matrices are plotted on the y-axis.

**Figure 3:** Model Response Curves for UV Adsorption at 260 nm



Note. The concentration response curves were generated based on Equation 1. conductivity response curve (left) reveals a negative effect of matrix conductivity on mRNA UV absorption, while the effect of concentration on  $A_{260}$  is linear, as expected based on Beer-Lambert law.

## Conclusion

- mRNA purification processes include matrices with a wide range of NaCl concentrations
- Matrix conductivity affects absorbance at 260 nm, potentially leading to errors in mass balance calculations
- Higher matrix conductivity results in lower absorbance values. Variations  $\leq 30\%$  can be observed in matrices containing 0–1 M NaCl
- A mathematical model is proposed to correct  $A_{260}$  measurements for the effect of matrix conductivity

## References

- Wilfinger, W. W., Mackey, K., & Chomczynski, P. (1997). Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques*, 22(3), 474–481. <https://doi.org/10.2144/97223ST01>