

Long-term Stability of Nicotinamide Cofactors in Common Aqueous Buffers: Implications for Cell Free Biocatalysis

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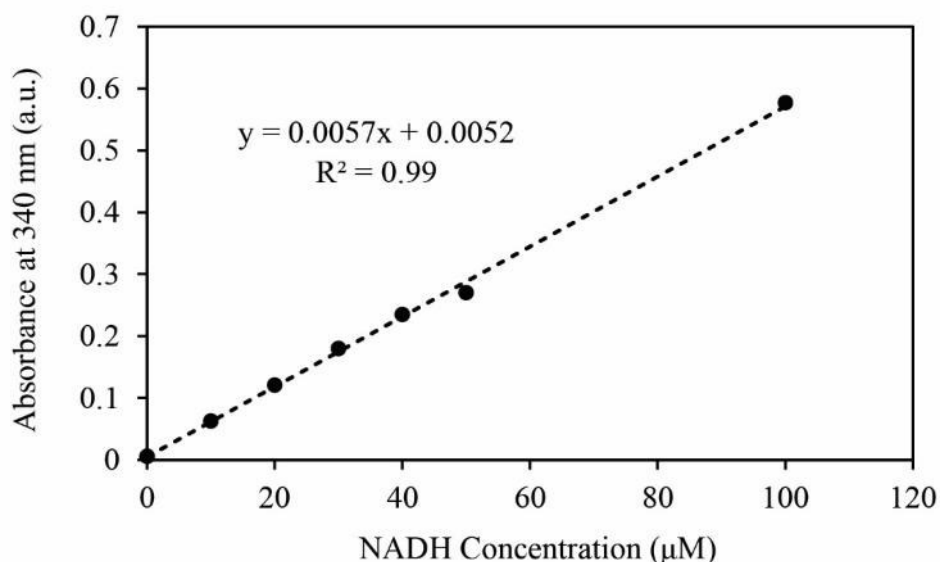


Figure S1. Calibration curve for absorbance at 340 nm vs. NADH concentration in HEPES buffer. Identical tests were completed in Tris and sodium phosphate buffers as well, resulting in minimally different slopes. These calibration curves were used to determine the NADH concentration used for stability curves shown in the following figures.

Supporting Information

Amino Acid Sequence of *Lactobacillus brevis* NADH oxidase (UniProt: Q8KRG4) with C-terminal His-tag:

MGKVTVVGCTHAGTFAIKQILAEHPDAEVTVYERNDVISFLSCGIALYLGGKVADPQGL
FYSSPEELQKLGANVQMNHNVLAIDPDQKTVTVEDLTSHAQTTESYDKLVMTSGSWPI
VPKIPGIDSDRVKLCKNWAHAQALIEDAKEAKRITVIGAGYIGAELAEAYSTTGHDVTLI
DAMDRVMPKYFDADFTDVIEQDYRDHGVQLALSETVESFTDSATGLTIKTDKNSYETD
LAILCIGFRPNTDLLKGKVDMAPNGAIITDDYMRSSNPDI FAAGDSAAVHYNPTHQNAYI
PLATNAVRQGILVGKNLVKPTVKYMGQTQSSSGLALYDRTIVSTGLTLAAAKQQGVNAE
QVIVEDNYRPEFMPSTEPVLMSLVFDPDTHRILGGALMSKYDVSQSANTLSVCIQNENTI
DDLAMVDMLFQPNFDRPFNYLNILAQAAQAKVAQSVNALEHHHHHH

<https://www.uniprot.org/uniprotkb/Q8KRG4/entry>

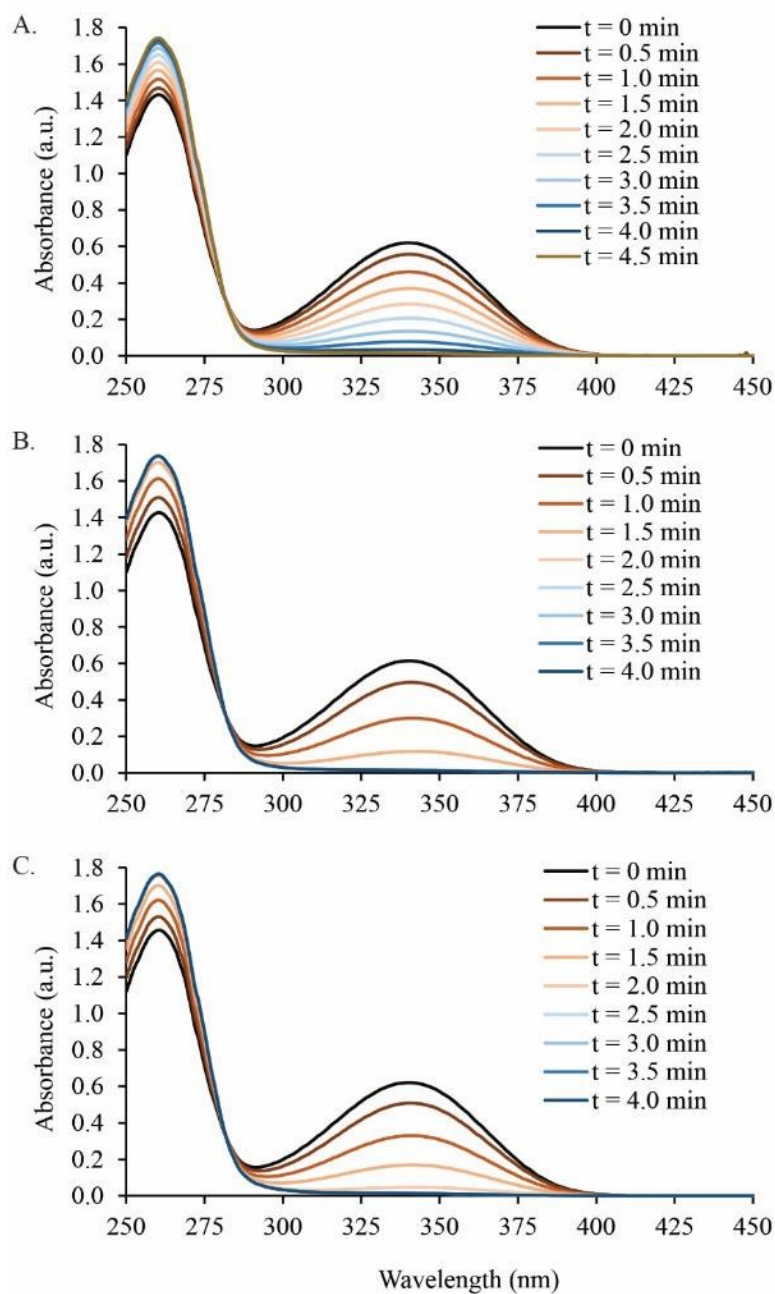


Figure S2. Examples (from dilution series study) of NOX consuming 100 μ M NADH as shown by a decrease in absorbance at 340 nm wavelength in A) Tris, B) HEPES, and C) phosphate buffers at pH 8.5 and buffer concentration of 50 mM.