

Title	Amplified detection of NAD ⁺ and NADH by the enzymatic cycling with the use of immobilized enzymes in a flow system
Sub Title	
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Publisher	共立薬科大学
Publication year	1992
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.37 (1992.) ,p.61- 61
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000037-0061

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Amplified Detection of NAD^+ and NADH by the Enzymatic Cycling with the Use of Immobilized Enzymes in a Flow System*

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The enzymatic cycling for the detection of NAD^+ and NADH in low level was carried out in the flow system with immobilized enzymes. Alcohol dehydrogenase and glutamate dehydrogenase immobilized on Sepharose 4B were used for the cycling reaction. The compound produced in the enzymatic cycling reaction was subjected to an enzymatic reaction to yield NADH, which was detected fluorometrically.

NAD^+ and NADH were amplified by a factor of 19.5 and 16.0, respectively, through the columns with immobilized enzymes. The calibration curves showed linear relationships over the range of three orders of sample concentrations ($0.01\text{--}10\ \mu\text{M}$). The detection limits of NAD^+ and NADH were both less than $0.2\ \text{pmol}$. The determination of NAD^+ (H) by the present method could be carried out in 8 min (in 6 min for the samples less than $1\ \mu\text{M}$).

* 本報告は *Anal. Lett.*, **25** (9), 1631—1641 (1992) に発表.