Enzymatic Assay of LEUCINE DEHYDROGENASE
(EC 1.4.1.9)

PRINCIPLE:

L-Leucine + ß-NAD $\xrightarrow{\text{Leucine Dehydrogenase}}$ a-Ketoisocaproate + ß-NADH

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 37°C, pH = 10.5, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Glycine with 200 mM Potassium Chloride Buffer, pH 10.5 at 37°C
(Prepare 100 ml in deionized water using Glycine Free Base, Prod. No. G-7126, and Potassium Chloride, Prod. No. P-4504. Adjust to pH 10.5 at 25°C with 1 M KOH.)

B. 20 mM L-Leucine Solution (Leu)
(Prepare 20 ml in Reagent A using L-Leucine, Prod. No. L-8000.)

C. 12.5 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Prod. No. N-7004 or dissolve the contents of one 20 mg vial of ß-Nicotinamide Adenine Dinucleotide, Stock No. 260-120, in the appropriate volume of deionized water. **PREPARE FRESH.**)

D. 25 mM Potassium Phosphate Solution, pH 7.2 at 37°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous Prod. No. P-5379. Adjust to pH 7.2 at 37°C with 1 M KOH.)

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REAGENTS: (continued)

E. Leucine Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 - 0.5 unit/ml of Leucine Dehydrogenase in cold Reagent D.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

	Test	Blank
Reagent B (Leu)	3.00	3.00
Reagent C (ß-NAD)	0.30	0.30

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Diluent)	------	0.05
Reagent E (Enzyme Solution)	0.05	------

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the $r\ A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r\ A_{340nm}/\text{min Test} - r\ A_{340nm}/\text{min Blank}}{(6.22)\ (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will convert 1.0 µmole of L-leucine to a-ketoiso-caproate per minute at pH 10.5 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 3.35 ml reaction mix, the final concentrations are 179 mM glycine, 179 mM potassium chloride, 18 mM L-leucine, 1.1 mM ß-NAD, 0.37 mM potassium phosphate and 0.005 - 0.025 unit leucine dehydrogenase.

REFERENCES:

Ohshima, T., Misono, H. and Soda, K (1978) *Journal of Biological Chemistry* **253**, 5719.

Soda K., Misono, H., Mori, K. and Sakato, H. (1971) *Biochemical and Biophysical Research Communication* **44**, 931.

NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.