

**Enzymatic Assay of  
LEUKOTRIENE D<sub>4</sub> HYDROLASE  
(EC 3.4.13.19)**

**PRINCIPLE:**

Gly-D-Phe + H<sub>2</sub>O  $\xrightarrow{\text{Leukotriene D}_4 \text{ Hydrolase}}$  D-Phe + Gly

Abbreviations used:

D-Phe = Phenylalanine

Gly = Glycine

**CONDITIONS:** T = 37°C, pH = 8.0, A<sub>214nm</sub>, Light path = 1 cm

**METHOD:** Stopped HPLC Analysis of Products

**REAGENTS:**

- A. 100 mM Tris HCl Buffer, pH 8.0 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 37°C with 1 M HCl.)
- B. 10 mM Gly-D-Phe Solution (Gly-D-Phe)  
(Prepare 1 ml in Reagent A using Gly-D-Phe, Sigma Prod. No. G-4879.)
- C. 2.4 mM D-Phenylalanine Solution (Phe Std)  
(Prepare 5 ml in Reagent A using D-Phenylalanine, Sigma Prod. No. P-1751. Initially prepare a stock solution containing 0.40 mg/ml. Further dilute to stock concentrations of (mg/ml): 0.30, 0.20, 0.10, and 0.050.)
- D. Leukotriene D<sub>4</sub> Hydrolase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.05 - 0.1 unit/ml in cold Reagent A.)
- E. 0.08% (w/v) Phosphoric Acid Solution (Buffer A)  
(Prepare 200 ml in deionized water using Phosphoric Acid, Sigma Prod. No. P-6560. Adjust to pH 2.5 with 1 M NaOH.)

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**REAGENTS:** (continued)

F. Acetonitrile (Buffer B)  
(Use Acetonitrile, Sigma Stock No. 27,071-7.)

**PROCEDURE:**

Step 1:

Pipette (in milliliters) the following reagents into suitable microcentrifuge tubes:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.08	0.09
Reagent D (Enzyme Solution)	0.01	-----

Equilibrate to 37°C. Then add:

Reagent B (Gly-D-Phe)	0.01	0.01
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Incubate for exactly 30 minutes at 37°C. Then terminate the reaction by heating at 100°C for 4 minutes. Microcentrifuge at 15,000 g for 10 minutes and then transfer 0.08 ml of the supernatant from both the Test and Blank to HPLC vials.

Step 2:

HPLC analysis of reaction products.

1. Column: Supelcosil LC-18, Supelco Catalog No. 5-8230, 4.6 x 150 mm, 5 µm particle size.

Mobile Phase	Time (min)		
	0	15	25
Buffer B (Reagent F)	4.5%	30%	30%
Buffer A (Reagent E)	95.5%	70%	70%

Pressure: 2150 PSI<sup>1</sup>, flow rate 1.5 ml/min, detection: 214nm, Sample volume injected: 20 µl.

3. Inject blank and standards of D-phenylalanine (Reagent C). A comparison can then be made between the standard curve of D-phenylalanine and D-phenylalanine generated from the sample reaction.

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**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of D-phenylalanine})(0.1)}{(0.01)(30)}$$

0.1 = Volume (in milliliter) of assay

0.01 = Volume (in milliliter) of enzyme used

30 = Time (in minutes) of assay

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

**UNIT DEFINITION:**

One unit will produce 1.0  $\mu$ mole of D-phenylalanine from Gly-D-Phe per min at pH 8.0 at 37°C.

**FINAL ASSAY CONCENTRATION:**

In a 0.10 ml reaction mix, the final concentrations are 100 mM Tris, 1 mM gly-D-phe, and 0.0005 - 0.001 unit of leukotriene D<sub>4</sub> hydrolase.

**REFERENCE:**

Littlewood, G.M., Hooper, N.M., and Turner, A.J. (1989) *Biochemical Journal* **257**, 361-367.

**NOTES:**

1. This pressure may vary.
2. This assay is based on the cited reference.
3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

**This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.**