Enzymatic Assay of
LEUKOTRIENE D₄ HYDROLASE
(EC 3.4.13.19)

PRINCIPLE:
Gly-D-Phe + H₂O  \( \text{Leukotriene D₄ Hydrolase} \)  \( \rightarrow \)  D-Phe + Gly

Abbreviations used:
D-Phe = Phenylalanine
Gly = Glycine

CONDITIONS:  \( T = 37^\circ C, \text{pH} = 8.0, A_{214nm}, \text{Light path} = 1 \text{ cm} \)

METHOD:  Stopped HPLC Analysis of Products

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 8.0 at 37\(^\circ\)C
(Prepare 100 ml in deionized water using Trizma Base, Adjust to pH 8.0 at 37\(^\circ\)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.)

C. 2.4 mM \( \text{D-Phenylalanine Solution (Phe Std)} \)
(Prepare 5 ml in Reagent A using \( \text{D-Phenylalanine} \), 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 D₄ 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. Adjust to pH 2.5 with 1 M NaOH.)
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REAGENTS:  (continued)

F. Acetonitrile (Buffer B)

PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.01</td>
<td>------</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (Gly-D-Phe)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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.

2. Mobile Phase

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>15</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer B (Reagent F)</td>
<td>4.5%</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>Buffer A (Reagent E)</td>
<td>95.5%</td>
<td>70%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Pressure: 2150 PSI, 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:

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

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 µmole 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 D₄ hydrolase.

REFERENCE:


NOTES:

1. This pressure may vary.

2. This assay is based on the cited reference.