**Microsomal Cytochrome P450 2B Testosterone Assay**

*Enzyme Mixture:*
Working stock of microsomes (1.5-2 mg/mL P450)
Use assay buffer to dilute frozen stock to working concentration.

*Substrate:*
Add 10 or 20 nmol of testosterone per tube.

**Assay Buffer**

<table>
<thead>
<tr>
<th>For 10 mL:</th>
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<tr>
<td>100 mM HEPES, pH 7.4</td>
<td>1 mL 1 M HEPES, pH 7.4</td>
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<tr>
<td>15 mM MgCl₂</td>
<td>75 uL 2 M MgCl₂, autoclaved</td>
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<tr>
<td>1 mM DTT</td>
<td>10 uL 1 M DTT</td>
</tr>
<tr>
<td>0.1-1 mM EDTA</td>
<td>2-20 uL 500 mM EDTA, autoclaved</td>
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<tr>
<td>ddH₂O to 10 mL</td>
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**Reaction mixture:**
Add the following to each tube containing substrate:
160 μL Assay buffer
20 μL microsomal P450
20 μL 10 mM NADPH
200 μL Total Volume

NADPH: 10 mM in H₂O (prepare fresh or use frozen stock)

**Procedure:**
1) Add Substrate to 12 X 75 mm or 13 X 100 mm borosilicate glass tube and evaporate solvent under gentle stream of N₂.
2) Add 160 μL Assay Buffer and 20 μL microsomal P450 to each tube and incubate for 5 min at 37 °C in sequential manner, i.e. place the tubes every 15 sec.
3) Initiate reaction by the addition of 10 mM NADPH. Add 20 μL NADPH to each tube in the same order sequentially every 15 sec and incubate for 10-20 min at 37 °C.

*For LC/MS:*
4) Terminate reaction with 1 mL acetonitrile by adding to each tube in the same order sequentially every 15 sec. Place on ice.
5) Vortex for 30 sec. to extract products.
6) Analyze by LC/MS.

*For GC/MS:*
7) Terminate reaction with 1 mL hexanes by adding to each tube in the same order sequentially every 15 sec.
8) Vortex for 30 sec. to extract products.
9) Analyze by GC/MS.
If yield is low, use 0.5 mL acetonitrile or hexanes for extraction. The hexanes may be evaporated and the sample reconstituted with an addition of 100 µL of hexanes to further concentrate the sample.

Solutions:

**NOTE: Use ultrapure (double distilled/milliQ pure) water for all solutions (ddH₂O)**

1 M HEPES, pH 7.4 (FW = 260.29)
130.145 g HEPES (Sigma #: H8651)
Add ddH₂O to 500 mL

2 M MgCl₂, autoclaved (FW = 203.30)
203.30 g MgCl₂ (EMD #: MX0045-4)
Add ddH₂O to 500 mL

1 M DTT, filtered (FW = 154.25)
1.5425 g DL-DTT (Sigma #: D0632)
Add ddH₂O to 10 mL
Freeze in 200 µL aliquots

0.5 M EDTA, autoclaved (FW = 372.24)
93.06 g EDTA (Fisher #: BP120)
Add ddH₂O to 500 mL

10 mM NADPH (FW = 833.4)
83.34 mg NADPH (Sigma #: N-6505)
Add ddH₂O to 10 mL
Freeze in 1 mL aliquots