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Hypertension ELISA (20-HETE) kit

Cat # 20H 1: ELISA kit for measuring 20-HETE in biological samples:

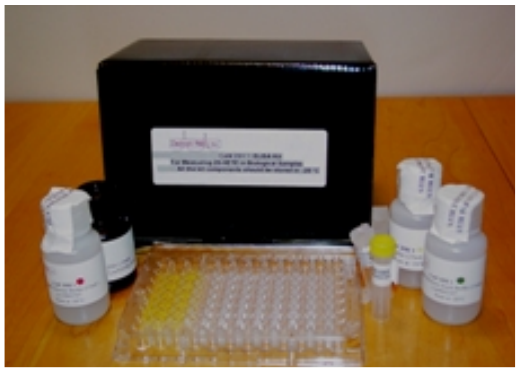
This competitive ELISA kit is for determination of 20-HETE (also known as 20-OH-AA) levels in biological samples. The specificity of the 20-HETE ELISA was investigated using authentic 20-HETE and a panel of fatty acids which, based on their structure, might be anticipated to compete with 20-HETE for binding to antibodies for 20-HETE. Anti-20-HETE did not cross-react with 14,15- and 11,12-DHETs, PGE_2 and showed almost no cross-reactivity even with structurally extremely similar arachidonic acid (AA), linoleic acid and linolenic acid as shown in the competitive ELISA analysis (see below). Considering that the only difference between 20-HETE and AA is an oxygen molecule, the specificity of the Detroit R&D 20-HETE ELISA is a surprise.

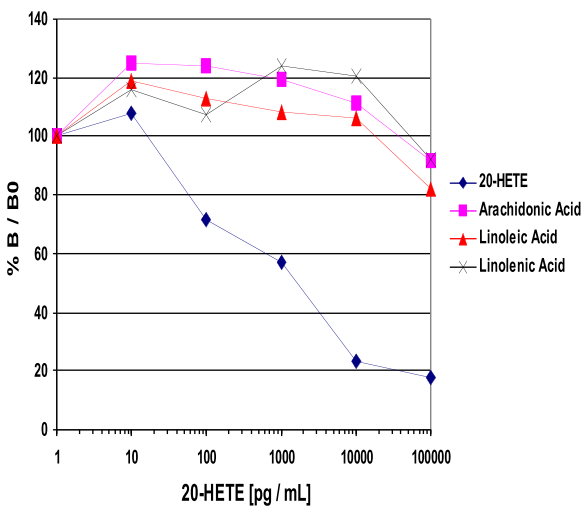
Human essential (1) and salt-sensitive (2,3) hypertensions were related to differential AA metabolism by cytochrome P450 (CYP) 4A which has AA ω-hydroxylase (20-HETE synthesis) activity. Increased circulating insulin inhibits 20-HETE synthesis in obese hypertensive subjects (4).

Each kit for triplicate analyses of up to 24 samples contains one 96 well plate, one tube of 20-HETE standard, one tube of 20-HETE-conjugated horseradish peroxidase (HRP), and buffers for sample and HRP dilutions, and plate washing.

Cat # 20H 11: 2X ELISA kits for measuring 20-HETE in biological samples:

Cat # 20H 21: 5X ELISA kits for measuring 20-HETE in biological samples:





[1]Capdevila et al. Alterations in the regulation of androgen-sensitive Cyp 4a monooxygenases cause hypertension. Proc. Natl. Acad. Sci. USA. 98, 5211, 2001.

[2]Laffer, C. L., Laniado-Schwartzman, M., Wang, M. H., Nasjletti, A. and Elijovich, F. Differential regulation of natriuresis by 20-hydroxyeicosatetraenoic acid in human salt-sensitive versus salt-resistant hypertension. Circulation 107, 574, 2003.

[3]Laffer, C. L., Laniado-Schwartzman, M., Wang, M. H., Nasjletti, A. and Elijovich, F. 20-HETE and furosemide-induced natriuresis in salt-sensitive essential hypertension. Hypertension 41, 703, 2003.

[4]Laffer, C. L., Laniado-Schwartzman, Nasjletti, A. and Elijovich, F. Differential regulation of natriuresis by 20-HETE and circulating insulin in essential hypertension with obesity. Hypertension 43, 388, 2004.

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Measurement of 14,15-DHET in plasma or serum

Extraction protocol

Step 1: Purification
1.8mL plasma adjusted with approximately 20µL acetic acid to pH 4 + 1.8mL ethyl acetate
Vortex
Centrifugation: t=10 min., T: 22°C, 2000rpm
Phase separation: upper phase = Ethyl acetate phase (lipoproteins) = organic phase
Interphase = proteins
Lower phase = aqueous phase
Collect upper phase (a)
2X: add equal volume ethyl acetate to lower phase after transferring lower phase with glass pipette to new tube, discard interphase (proteins), centrifuge and collect again upper phase

Step 2: Evaporation
Approximately 4mL ethyl acetate phase (a)
Dry in Speedvac = sediment (b)

Step 3: Saponification: (to cleave fatty acid from glycerol backbone)
20% KOH Solution: 1mL of 2M KOH + 4mL Methanol (MeOH) (final concentration KOH = 0.4N)
Sediment (b) + 2 mL 20% KOH
Vortex
Incubate: t = 1 h, T = 50° C
= aqueous solution (c)

Step 4: Re-extraction
2mL aqueous solution (c) + 3 mL H_2O
Adjust pH with 20% formic acid to pH 7.4 (132µL)
Add 2mL ethyl acetate
(1 part aqueous solution (c) + 1 part ethyl acetate)
Vortex
Centrifuge: t = 10 min., T = 22°C, 2000 rpm
Repeat procedure with ethyl acetate 2X (equal volume ethyl acetate per sample)
Collect upper phase (with saponified lipids)
Check pH of upper phase (should be 7.4)

Step 5: Evaporation
6mL ethyl acetate phase, upper phase (d)
Dry in speedvac = sediment (e)
Store sediment (e) at -20° C
For ELISA assay, dissolve sediment in 20µL ethanol, then add 130µL ELISA-sample buffer.
Check pH (should be 7.4 after adding ethanol)

Step 6: Competitive 14,15-DHET - ELISA
150µL Probe: a) Dilution 1:5, (i.e., 80µL sample + 320µL ELISA-sample buffer
b) For calculating concentration, consider dilution factor (in this case 1.875, i.e., 80µL sample from the 150µL total sample volume)