



## Hypertension/Stroke 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 or PGE<sub>2</sub> and showed almost no cross-reactivity even with structurally extremely similar arachidonic acid (AA), linoleic acid and linolenic acid (see plot below).

Hypertension was caused by AA  $\omega$ -hydroxylase (20-HETE synthesis) activity of cytochrome P450 (CYP) 4A, 4F<sup>4</sup>, 1B<sup>17,8</sup> or kidney androgen-regulated protein (KAP)<sup>2,16</sup>. CYP4F2 genetic variants, which increased urinary 20-HETE secretion, were correlated with the risk for hypertension in a Chinese population<sup>1,13</sup>. Urinary 20-HETE levels of two-kidney, one-clip (2K1C) rats were higher than control rats<sup>12</sup>. Co-inhibition of 20-HETE and DHET formation abolished angiotensin II hypertension in mice<sup>11</sup>, suggesting new generation hypertension drug development opportunity. 20-HETE was a clinical marker of post-transplant allograft function<sup>3</sup> and increased after cerebral ischemia, which induced brain injury due to its vasoconstrictive activity<sup>18</sup>. Recent studies reported that increased 20-HETE synthesis reduced cerebral blood flow<sup>20</sup> and vasodilatory effect of eNOS is a result of suppressed 20-HETE synthesis in brain slices<sup>21</sup>. The interplay of circadian clock, 20-HETE pathway and renal sodium handling was studied in mice<sup>17</sup>. 20-HETE formation and CYP23 expression were all decreased by N-palmitoylethanolamide treatment in SHR rats<sup>25</sup>. Thus, 20-HETE is a new biomarker and therapeutic target for hypertension<sup>19</sup>, stroke and even cancer<sup>24</sup>. A sharp decrease in 20-HETE levels in blood, urine and tissue is a clinical marker of hypotension and septic shock. High glucose fed rat proximal tubular cells elevated CYP4A expression and 20-HETE formation and activated the mTOR/p70S6Kinase pathway which plays a major role in diabetic nephropathy.<sup>26</sup>

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

### Related Products

#### Hypertension/Stroke ELISA kits:

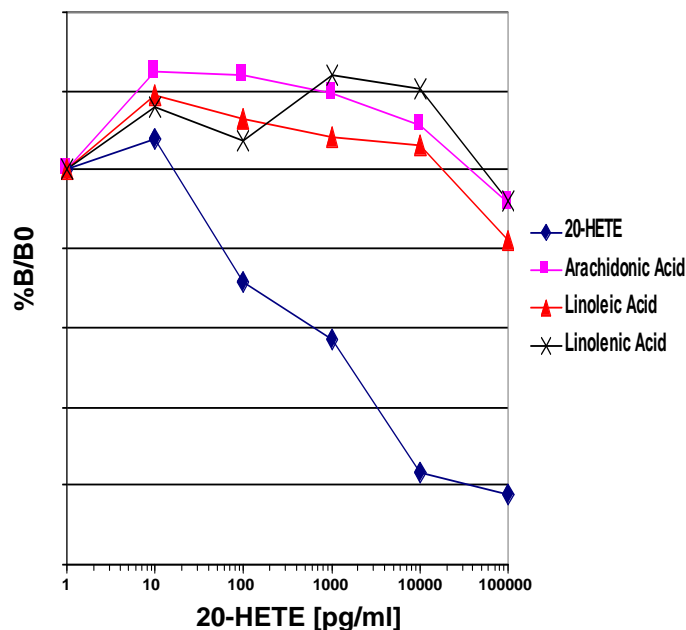
- 20-HETE B-glucuronide ELISA
- 14,15-DHET Hypertension/Stroke ELISA
- 11,12-DHET Hypertension/Stroke ELISA
- 12(S)-HETE Hypertension/Stroke ELISA
- 15(S)-HETE Hypertension/Stroke ELISA

#### Oxidative Stress ELISA Kit:

- 8-isoprostane ELISA

#### Hypertension/Stroke Antibodies:

- Rat: CYP2C23, CYP2C11, CYP2C, CYP4A, sEH
- Human: CYP1B1, CYP2C8/9, sEH, CYP4A



## References

1. Liu et al. Association of a functional cytochrome P450 4F2 haplotype with urinary 20-HETE and hypertension. *J. Am. Soc. Nephrol.* 19, 714-721, 2008.
2. Meseguer et al. Kidney androgen-regulated protein transgenic mice show hypertension and renal alterations mediated by oxidative stress. *Circulation.* 119, 1908-1917, 2009.
3. Dolegowska, B., Blogowski, W., Domanski L. Is it possible to predict the early post-transplant allograft function using 20-HETE measurements? A preliminary report. *Transpl Int.* 22, 546-553, 2009.
4. Liu et al. Overexpression of cytochrome P450 4F2 in mice increases 20-hydroxyeicosatetraenoic acid production and arterial blood pressure. *Kidney. International.* 75, 1288-1296, 2009.
5. Wang et al. Selective inhibitors of CYP2J2 related to terfenadine exhibit activity strongly against human cancers **in vitro** and **in vivo**. *J. Pharmacol. Exp. Ther.* 329, 908-918, 2009.
6. Cuez, Malik, Tunctan et al. A synthetic analogue of 20-HETE, 5,14-HEDGE, reverses endotoxin-induced hypotension via increased 20-HETE levels associated with decreased iNOS protein expression and vasodilator prostanoid production in rats. *Basic Clin. Pharmacol. Toxicol.* 106, 378-388, 2010.
7. Malik et al. 2,3',4,5'-Tetramethoxystilbene prevents deoxycorticosterone-salt-induced hypertension: contribution of cytochrome P-450 1B1. *Am. J. Physiol. Heart Circ. Physiol.* 299, H1891-H1901, 2010.
8. Malik et al. Cytochrome P450 1B1 contributes to angiotensin II-induced hypertension and associated pathophysiology. *Hypertension* 56, 667, 2010.
9. Tunctan et al. Contribution of vasoactive eicosanoids and nitric oxide production to the effect of selective cyclooxygenase-2 inhibitor, NS-398, on endotoxin-induced hypotension in rats. *Basic Clin. Pharmacol. Toxicol.* 107, 877-882, 2010.
10. Imaizumi et al. L-4F differentially alters plasma levels of oxidized fatty acids resulting in more anti-inflammatory HDL in mice. *Drug Metab. Letters*, 4, 139-148, 2010.
11. Cervenka, Kramer, Falck, Imig, Hammock et al. Combined inhibition of 20-HETE formation and of EET degradation attenuates hypertension and hypertension-induced end-organ damage in Ren-2 transgenic rats. *Clinical Science* 118, 617-632. 2010.
12. Cervenka, Kramer, Falck, Imig et al. Intrarenal CYP-450 metabolites of arachidonic acid in the regulation of the nonclipped kidney function in two-kidney, one-clip Goldblatt hypertensive rats. *J Hypertens* 28, 582-593, 2010.
13. Hu, Wang et al. Peripheral and central augmentation indexes in relation to the CYP4F2 polymorphisms in Chinese. *J Hypertens* 29, 501-508, 2011.
14. Buharalioglu et al. Piroxicam reverses endotoxin-induced hypotension in rats: contribution of vasoactive eicosanoids and nitric oxide. *Basic Clin Pharmacol Toxicol* 186–194, 2011.
15. Na-Bangchang et al. Study on the association between environmental cadmium exposure, cytochrome P450-mediated 20-HETE, heme-oxygenase-1 polymorphism and hypertension in Thai population residing in a malaria endemic areas with cadmium pollution. *Environ Toxicol Pharmacol* 31, 416-426, 2011.
16. Grande et al. Increased oxidative stress, the renin-angiotensin system, and sympathetic overactivation induce hypertension in kidney androgen-regulated protein transgenic mice. *Free Radical Biology & Medicine*, 51: 1831-1841. 2011.
17. Nikolaeva et al. The circadian clock modulates renal sodium handling. *J. Am. Soc. Nephrol.* 23: 1019-1026, 2012.
18. Onoe et al. Increase of 20-HETE synthase after brain ischemia in rats revealed by PET study with (11)C-labeled 20-HETE synthase-specific inhibitor. *J Cereb Blood Flow Metab* 32, 1737-1746, 2012.
19. Dong H. Metabolomic profiling of lipids for biomarker discovery. *Biochem Anal Biochem* 1, 5, 2012.
20. Fordsmann JC, Ko RW, Choi HB, Thomsen K, Witgen BM, Mathiesen C, Lønstrup M, Piilgaard H, MacVicar BA, Lauritzen M. Increased 20-HETE synthesis explains reduced cerebral blood flow but not impaired neurovascular coupling after cortical spreading depression in rat cerebral cortex. *J Neurosci* 33, 2562-2570, 2013.
21. Stobart JL, Lu L, Anderson HD, Mori H, Anderson CM. Astrocyte-induced cortical vasodilation is mediated by D-serine and endothelial nitric oxide synthase. *PNAS USA.* 110, 3149-3154, 2013.
22. Tunctan 20-HETE mimetics or inhibitors in the treatment of cancer patients with sepsis and septic shock. *International Journal of Cancer Studies & Research (IJCR)* 2:101, 2013.
23. Tunctan et al. NS-398 reverses hypotension in endotoxemic rats: Contribution of eicosanoids, NO, and peroxynitrite. *Prostaglandins & other Lipid Mediators* 104–105 93–108, 2013 .
24. Alexanian, A, Sorokin A. Targeting 20-HETE producing enzymes in cancer--rationale, pharmacology and clinical potential. *Onco Targets and Therapy* 6: 243-255, 2013.
25. Raso et al. N-Palmitoylethanolamide protects the kidney from hypertensive injury in spontaneously hypertensive rats via inhibition of oxidative stress. *Pharmacological Research* 76: 67-76, 2013.
26. Eid et al. 20-HETE and EETs in diabetic nephropathy: A novel mechanistic pathway. *PLOS One* 8: e70029, 2013
27. Ross et al. Proinflammatory high-density lipoprotein results from oxidized lipid mediators in the pathogenesis of both idiopathic and associated types of pulmonary arterial hypertension. *Pulmonary circulation* 5: 640-648, 2015

**Specificity of anti-20-HETE ELISA**

<b>Eicosanoids</b>	<b>% Binding of control</b>
20-HETE	100.00
Arachidonic Acid	<0.02
Linoleic Acid	<0.02
Linolenic Acid	<0.02
15-HETE	<0.02
14,15-DHET	<0.02
11,12-DHET	<0.02
PGE <sub>2</sub>	<0.02