



Metro Center for High Technology Bldg.
2727 Second Ave. Suite 4113
Detroit, MI 48201
Phone: (313) 961-1606; Fax: (313)963-7130
Email: info@DetroitRandD.com
Web: www.DetroitRandD.com

20-HETE / β -Glucuronidase ELISA Kit Cell-Based ELISA Assays

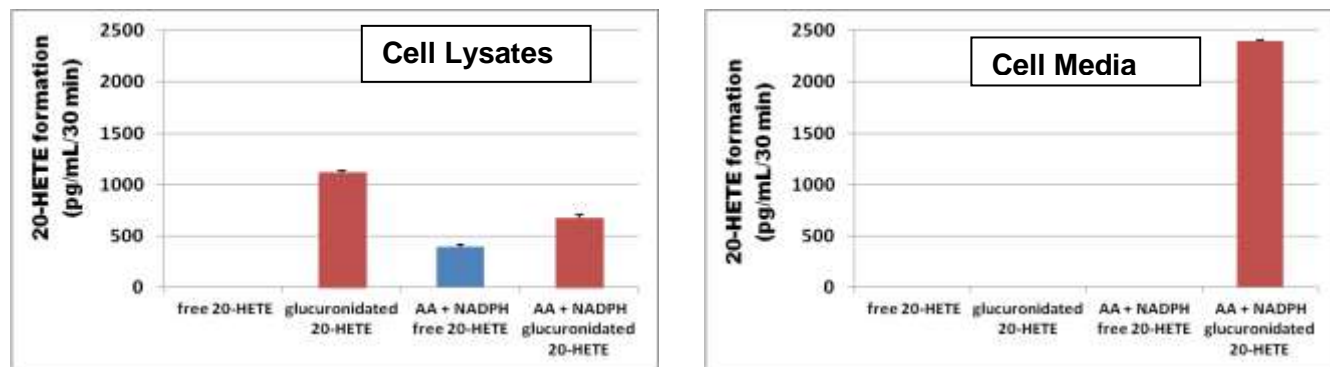
Cat # 20HG 1: ELISA kit for measuring glucuronidated 20-HETE formation: **1X=\$290**

This competitive ELISA kit is for determination of free and glucuronidated 20-HETE levels in cell extracts and cell media. The kit is similar to our 20-HETE ELISA Kit (Cat # 20H 1) but also contains the beta-glucuronidase enzyme. Each 96-well plate is sufficient for triplicate analyses of up to 12 free and 12 glucuronidated samples.

Buy in Quantity and Save! 2-3 kits \$ 276 each; 5-9 kits \$262 each; 10 or more kits \$247

Arachidonic acid (AA)-dependent 20-HETE formation activity of normal rat kidney (NRK) cells

ELISA measurements of 20-HETE formation in 100 μ L of media (1/10th total volume) and cell lysates following incubation of 5×10^4 cells for 30 min with or without 100 μ M AA and 1 mM NADPH. See a protocol on **page 2**.



20-HETE ELISA 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.* 2009 22:546-553
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. 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. 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.
9. 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.
10. Chabova et al. Combined inhibition of 20-hydroxyeicosatetraenoic acid formation and of epoxyeicosatrienoic acids degradations attenuates hypertension and hypertension-induced end-organ damage in Ren-2 transgenic rats. *Clinical Science* 118, 617-632 (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. [Tunçtan 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.

Protocol for Glucuronidated 20-HETE Measurement

MATERIALS

1. Dissolve 8 mg of beta-glucuronidase (provided) in 8 mL of 1 M citric acid, pH 5.5.
2. 20-HETE ELISA Kit Cat# 20H1 (provided)

PROTOCOL

- A. Measurement of free 20-HETE in cell lysates and media (Based on 5 X 10⁴ cells/mL reaction mixture):**
For free 20-HETE in cell lysates, extract lysates with ethyl acetate as described below, dry and dissolve in 20 µL ethanol and then dilute the ethanol dissolved sample with sample dilution buffer to 0.5 mL and apply to ELISA (100 µL/well). For free 20-HETE in media, dilute media with sample dilution buffer and apply to ELISA (100 µL/well). A 4X dilution is recommended.
- B. Measurement of glucuronidated 20-HETE in cell lysates (Based on 5 X 10⁴ cells/mL reaction mixture)**
- (i) Extraction**
1. Collect cells from reaction mixture by centrifuging at 300 x g.
 2. Suspend cell pellet in 1.0 mL media, vortex vigorously, centrifuge at 300 x g, remove media leaving about 300 µL of media behind. Vortex vigorously.
 3. Add 1.0 mL ethyl acetate to pellet and vortex vigorously.
 4. Centrifuge at high speed to separate aqueous and organic layers. (If performing multiple extractions with ethyl acetate, combine the ethyl acetate fractions and proceed to next step).
 5. Remove ethyl acetate layer and dry using a speed vac or under a gentle stream of nitrogen or argon.
 6. Re-suspend dried extract in 20 µL ethanol, dissolve
 7. For β-glucuronidase digestion, bring up to 1.0 mL with sample dilution buffer and pipet 0.5 mL into two tubes.
- (ii) β-Glucuronidase digestion**
- 1 Add 200 uL of the beta-glucuronidase solution, pH 5.5, to one tube (see MATERIALS), final pH 5.5.
 - 2 Add 200 uL of the 1 M citric acid solution to the second tube (no enzyme).
 - 3 Incubate both tubes at 37°C for 3 hours.
 4. If performing the ELISA immediately then go onto step (iii). If not, freeze immediately.
- (iii) ELISA**
1. Follow instructions for ELISA kit. Samples may need to be diluted prior to adding to the ELISA plate. A 4X dilution is recommended.
 2. To calculate the amount of glucuronidated 20-HETE, subtract the value of the non-enzyme time point from the 3- hour time point.
- C. Measurement of glucuronidated 20-HETE in Media (Note—Extraction is not necessary).**
- (i) β-Glucuronidase digestion**
1. Pipet 0.5 mL of the extracellular media into two tubes.
 2. Add 200 uL of the beta-glucuronidase enzyme to one tube (see MATERIALS), final pH 5.5.
 3. Add 200 uL of the 1 M citric acid solution to the second tube (no enzyme).
 4. Incubate both tubes at 37°C for 3 hours. If performing the ELISA immediately then go onto step (iii). If not, freeze immediately
- (ii) ELISA**
1. Follow instructions for ELISA kit. Samples may need to be diluted prior to adding to the ELISA plate. A 4X dilution is recommended.
 2. To calculate the amount of glucuronidated 20-HETE, subtract the value of the non-enzyme sample from the 3 hour time point with enzyme.

Other Hypertension & Oxidative Stress ELISAs

20-HETE ELISA (Cat.# 20H1)

14,15-DHET/EET ELISA (Cat# DH 2)

sEH Toxicant & Drug Candidate Screening (Cat# SH 1)

11,12-DHET/EET ELISA Cat.# DH 5)

8-Isoprostane Oxidative Stress ELISA (Cat.# 8iso1)

Page 2

Metro Center for High Technology Bldg.
2727 Second Ave. Suite 4113
Detroit, MI 48201
Phone: (313) 961-1606; Fax: (313)963-7130
Email: info@DetroitRandD.com
Web: www.DetroitRandD.com