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20-HETE / β-Glucuronidase ELISA Kit

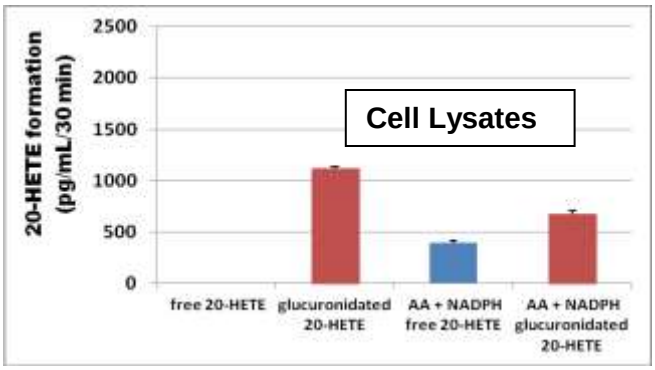
Cell-Based ELISA Assays

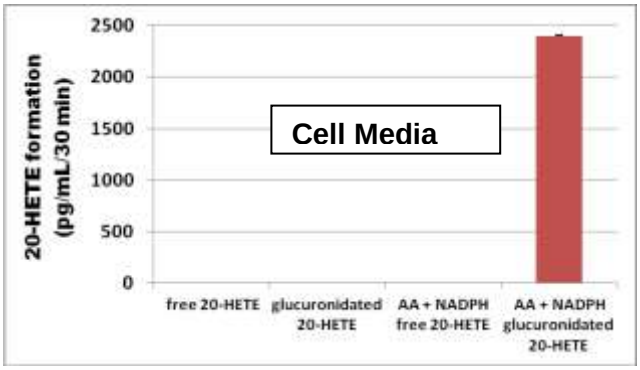
Cat # 20HG 1: ELISA kit for measuring glucuronidated 20-HETE formation

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.

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

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





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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 10^4 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 10^4 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.

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