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14,15-DHET Human Urine Test Kit

Cat # DH 3: ELISA kit for measuring 14,15-DHET in urine: $290

This competitive ELISA kit is for determination of free and glucuronidated 14,15-DHET levels in urine. The 14,15-DHET is a representative metabolite of soluble epoxide hydrolase-mediated metabolism of EETs, which are generated by arachidonic acid epoxygenase activity of cytochromes P450. 14,15-DHET level exhibited strong positive correlation with hypertension in rat and human and brain injury and stroke in rodents. Human urine and blood 14,15-DHET levels were measured using the 14,15-DHET ELISA kit. Increased 14,15-DHET levels of human cells as detected by the 14,15-DHET ELISA were indicative of the neoplastic and metastatic phenotype of carcinoma cells.

A) Free 14,15-DHET Measurement

Simple Assay : Urinary DHET can be measured without ethyl acetate extraction. Just dilute 4 to 10- fold and apply sample to ELISA plate for measurement of free DHET

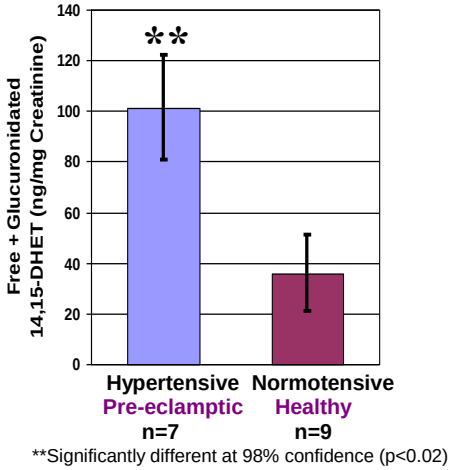
B) Glucuronidated 14,15-DHET Measurement:

This method is for determining the level of glucuronidated 14,15-DHET in urine after digestion of the molecule with glucuronidase. The ELISA antibody does not recognize the glucuronidated form of the 14,15-DHET molecule. Following digestion , any glucuronidated 14,15-DHET is converted to free 14,15-DHET which can be detected by the ELISA antibody.

C) Total 14,15-DHET Measurement (= A) Free + B) Glucuronidated)

Buy in Quantity and Save! 2-3 kits $276 each; 5-9 kits $261 each; 10 or more kits $247 each

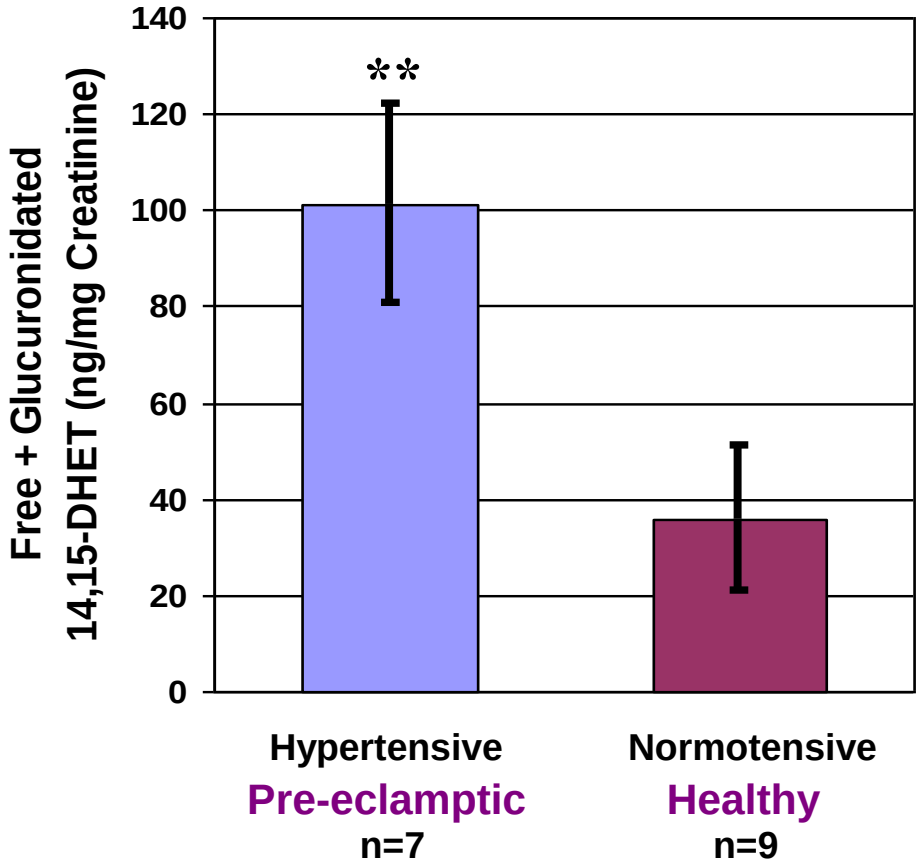
Each kit is for triplicate analyses of up to 24 free 14,15-DHET measurements and 12 glucuronidated 14,15-DHET measurements. The kit contains one 96 well plate, one tube of 14,15-DHET standard, one tube of 14,15-DHET-conjugated horseradish peroxidase (HRP), glucuronidase and buffers for glucuronidase enzyme and sample and HRP dilutions, and plate washing.



**Significantly different at 98% confidence (p<0.02)

14,15-DHET Human Clinical Data

ELISA Test with Coded Urine from Pregnant Women



Urinary 14,15-DHET levels of pregnancy-induced hypertensive (preeclamptic) women were significantly different at 98% confidence ($p<0.02$) from healthy pregnant women. **No false negative.**

Thus, the 14,15-DHET ELISA is useful for detection of soluble epoxide hydrolase- and/or UDP-glucuronosyl transferase-dependent hypertensions and development of new hypertension drugs.

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Recovery Rates of DHETs after Ethyl Acetate Extraction: Human Urine Studies

The recovery rate of 14,15-DHET after ethyl acetate extraction has been proven to be ~100% by 2 different methods of experiments carried out at our laboratory using human urine samples spiked with 14,15-DHET. They are (1) isotopic method and (2) ELISA. Our results demonstrated that with urine samples, and most likely with other biological samples, the ethyl acetate extraction has a minimal effect on the ELISA result.

1. Isotopic Method

Human urine samples were spiked with ^{3}H 14,15-DHET (55,660 cpm) or ^{3}H 8,9-DHET (13,899 cpm) and acidified to pH 3.0 and extracted into an equal volume of ethyl acetate. Extracts and remaining fractions were counted by a liquid scintillation counter. The counts taken by a liquid scintillation counter, demonstrated ~100 % recovery after 1X extraction (56,690 cpm).

Alternatively, the spiked urine samples were acidified to pH 3.0 and applied to C-18 solid phase extraction column. The column was first washed with 10% acetonitrile in water, pH 3.0, followed by washing with n-heptane. The bound DHETs were eluted with 1 ml ethyl acetate: heptane (1:1). Elution of DHETs with 1 ml ethyl acetate: heptane (1:1) was repeated until radioactivity of the eluates was low. The recovery rate with the C-18 solid phase extraction was low.

Table 1. Recovery rates of DHETs spiked in human urine after ethyl acetate extraction**: Isotopic Method.**

Treatment	[^{3}H] 14,15-DHET		[^{3}H] 8,9-DHET	
	CPM	%	CPM	%
None	55,660	100	13,899	100
C18 Solid Phase Extraction				
Flow-Through	1,241 ± 343	22.3 ± 0.6	260 ± 38	1.9 ± 0.3
1st Elution	22,544 ± 784	40.5 ± 1.4	5,294 ± 1,403	38.1 ± 10.1
2nd Elution	14,247 ± 951	25.6 ± 1.7	1,928 ± 465	13.9 ± 3.3
3rd Elution	1,101 ± 73	2.0 ± 0.1	138 ± 34	1.0 ± 0.2
Liquid Phase Extraction with Ethyl Acetate				
Extracts	56,690 ± 850	101.9 ± 1.5	13,155 ± 334	94.6 ± 2.4
Remaining Fractions	5,342 ± 2,291	9.6 ± 4.1	852 ± 182	6.1 ± 1.3

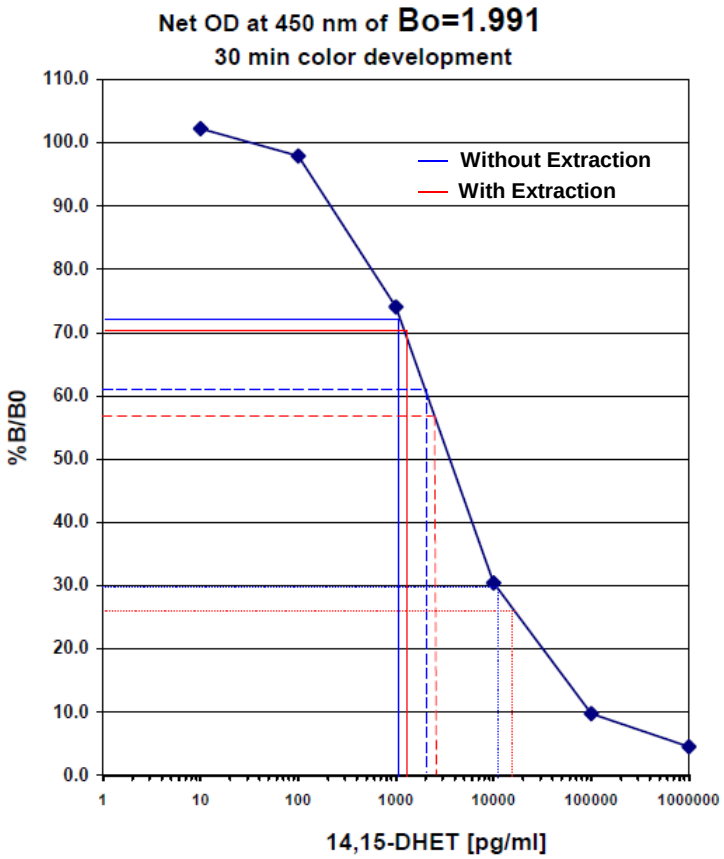
2. ELISA

An ELISA with and without ethyl acetate extraction using 4-fold diluted human urine spiked with various levels of 14,15-DHET was carried out using "Detroit R&D 14,15-DHET Hypertension ELISA" to verify our previously obtained result with ^{3}H 14.15-DHET spiked in human urine samples. The results are shown in **Table 2** and **Fig. 1**.

Table 2. Recovery rates of 14,15-DHET spiked in human urine samples after ethyl acetate extraction: **ELISA.**

Spiked Concentration (ng/ml)	Without Extraction				With Extraction			
	Net OD (N=3)	%B/Bo: Bo= 1.991	Experimental Concentration (ng/ml)	Theoretical Concentration (ng/ml)	Net OD (N=3)	%B/Bo: Bo= 1.991	Experimental Concentration (ng/ml)	Theoretical Concentration (ng/ml)
0 (Diluted Urine Only)	1.408	70.7	1.00	Not Applicable	1.437	72.2	1.00	Not Applicable
0.01	1.508	75.7	0.90	1.01	1.498	75.2	0.90	1.01
0.10	1.482	74.4	0.95	1.10	1.482	74.4	0.95	1.10
1.00	1.137	57.1	2.50	2.00	1.225	61.5	2.00	2.00
10.00	0.527	26.5	15.00	11.00	0.598	30.0	11.00	11.00
100.00	0.196	9.8	100.00	101.00	0.220	11	100.00	101.00
1000.00	0.113	5.7	1000.00	1001.00	0.104	5.2	1000.00	1001.00

Fig. 1. A 14,15-DHET standard curve used for quantitation of 14,15-DHET levels in diluted human urine samples spiked with various concentrations of 14,15-DHET.



The effect of ethyl acetate extraction of the 4-fold diluted human urine samples on the recovery rate was minimal: The 14,15-DHET level for the 4-fold diluted urine was ~1 ng/ml (~4 ng/ml without dilution of the urine) for without or with extracted samples. The levels were calculated from ODs of 1.408 and 1.437 (%B/Bo=70.7 and 72.2, respectively) for without and with extraction, respectively, using a standard graph obtained without addition of urine samples (**Fig. 1**).

When 1 ng/ml 14,15-DHET was spiked to the 4-fold diluted, ~1 ng/ml urine samples, the ODs were obtained by ELISA as 1.137 and 1.225 (%B/Bo=57.1 and 61.5, respectively) for without and with extraction, respectively (**Table 2**). The experimentally obtained 14,15-DHET levels of the 1 ng/ml 14,15-DHET spiked to 4-fold diluted urine samples were 2.5 ng/ml and 2 ng/ml for results obtained without and with extraction, respectively, which were close to expected (theoretical) value (1 ng/ml from the diluted urine + 1 ng/ml of spiked 14,15-DHET = 2 ng) (**Fig. 1**. the standard graph marked with 14,15-DHET levels for urine samples with and without spiked 14,15-DHET).

The 14,15-DHET levels of the 10 ng/ml 14,15-DHET spiked to 4-fold diluted, ~1 ng/ml urine samples were 15 ng/ml and 11 ng/ml for results obtained without and with extraction, respectively, which were close to the expected value (11 ng/ml) (**Fig. 1.** the standard graph marked with 14,15-DHET levels for urine samples with and without spiked 14,15-DHET. Also see **Table 2**).

The 14,15-DHET levels of the 100 ng/ml 14,15-DHET spiked to 4-fold diluted ~1 ng/ml urine samples were 100 ng/ml for results obtained without and with extraction, respectively which were close to expected (theoretical) value (101 ng/ml) (**Table 2**).

The detailed urine pretreatment method for ELISA:

The 2 µg/2 µl 14,15-DHET stock solution was serially diluted with 4-fold diluted urine to obtain various concentrations of spiked urine samples (total volume, 1.8 ml).

Acetic acid (20 µl) was added to the sample and extracted (1X) with 1.8 ml ethyl acetate. The mixture was vortexed and centrifuged (t=10 min., T: 22°C, 2000 rpm) to collect the upper phase (ethyl acetate phase: lipoproteins) which was dried using Speedvec. The dried sample was dissolved with 10 µl DMF and reconstituted to 1.8 ml with sample dilution buffer to be applied to "Detroit R&D 14,15-DHET Hypertension ELISA".