Exposure

Pyrene (HOP-G)

1-Hydroxypyrene Glucuronide ELISA Kit

Information for use

A product of Bioventix

1-Hydroxypyrene Glucuronide ELISA Kit

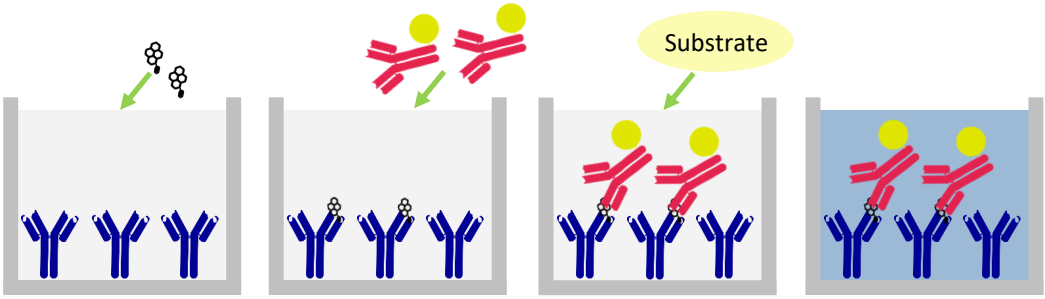
Description:

Enzyme-linked immunosorbent assay kit for the detection of 1-hydroxypyrene glucuronide (HOP-G) in urine samples. Sufficient for 40 samples run in duplicate.

Pyrene is a polycyclic aromatic hydrocarbon (PAH) commonly formed during the incomplete combustion of organic matter. When pyrene enters the body it is metabolised to form 1-hydroxypyrene (1-HOP) and subsequently HOP-G for urinary secretion. HOP-G is therefore an important biomarker for human exposure to airborne PAH pollutants.

Note: Any samples reading higher than the standard curve can be repeated with further dilution in a second assay.

Assay Principal:



A primary antibody to HOP-G is immobilized to the surface of the microplate wells. Urine samples containing HOP-G are incubated with the immobilised primary antibody. A second antibody with specificity to the immobilised antibody-HOP-G complex is then added, this second antibody is conjugated to an enzyme. Substrate addition initiates an enzyme reaction and a colour change, the colour change is then quantified.

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Kit Contents:

- Pre-coated and blocked immunosorbent 96-well plate (x1)
- HOP-G positive control (1.5 mL at 2000 pg/mL)
- Diluent Buffer (13 mL)
- Conjugate solution (13 mL)
- Substrate solution (13 mL)
- Stop solution (13 mL)
- 10 x Wash buffer concentrate (55 mL)

Storage Conditions: Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C).

Materials required but not provided:

- Adjustable single channel pipettes and disposable tips
- Adjustable multichannel pipettes and disposable tips
- Microplate reader capable of reading at 450 nm
- Multichannel pipette reservoir
- Deionised water

Sample Requirements:

- Urine samples should be centrifuged for 1 min at 17xG before dilution to remove precipitates
- Urines samples for long term storage should be kept at –80°C
- Urine samples can be stored short term at 2-8°C with the addition of preservative, e.g. Proclin

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Preparation of reagents:

Prepare 1 x wash buffer solution using 10 x wash buffer concentrate and deionised water. Note: Warm 10 x concentrate to room temperature to fully dissolve precipitated salt before diluting.

Prepare HOP-G standard curve following the table below.

Vial	Volume of Diluent	Volume and Source of HOP-G	Final HOP-G concentration
A	250 µL	250 µL from HOP-G stock	1000 pg/mL
B	215 µL	187 µL from vial A	464 pg/mL
C	215 µL	187 µL from vial B	215 pg/mL
D	215 µL	187 µL from vial C	100 pg/mL
E	215 µL	187 µL from vial D	46.4 pg/mL
F	215 µL	187 µL from vial E	21.5 pg/mL
G	215 µL	187 µL from vial F	10 pg/mL
H	215 µL	0	0 pg/mL

Assay Protocol:

1. Dilute samples as suggested in the 'Recommended dilutions' section.
2. Apply 100µL/well in duplicate the pre-prepared HOP-G standard curve from vials A-H to rows A-H in columns 1 and 2.
3. Apply samples, 100µL/well in duplicate. Incubate for 1 hour at room temperature.
4. Wash the plate 3 times, 300 µL/well, with 1 x Wash Buffer.
5. Apply Conjugate Solution, 100µL/well. Incubate for 1 hour at room temperature.
6. Wash the plate 9 times, 300 µL/well, with Wash Buffer.
7. Apply Substrate, 100µL/well. Incubate for 1 hour at RT.
8. Apply Stop Solution, 100µL/well.
9. Read plate/s at 450nM.

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Recommended dilutions and expected readings:

The accurate measuring range of the assay is between 22 and 220 pg/mL, use the following table to determine the appropriate dilutions for the type of samples being tested:

Sample Type	Expected [HOP-G]	Recommended Dilution
Non-Smoker, Not occupationally exposed	< 500 pg/mL	1 in 3 (1 part sample plus 2 parts diluent)
Smoker, Not occupationally exposed	500 - 1500 pg/mL	1 in 10 (1 part sample plus 9 parts diluent)
Occupationally Exposed	> 1500 pg/mL	1 in 30 (1 part sample plus 29 parts diluent)

Using the Result Template:

If you will be using the provided Microsoft Excel Result Template, the following plate layout should be adhered to.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	1000	Sample 1	Sample 1	Sample 2	Sample 2	Sample 3	Sample 3	Sample 4	Sample 4	Sample 5	Sample 5
B	464	464	Sample 6	Sample 6	Sample 7	Sample 7	Sample 8	Sample 8	Sample 9	Sample 9	Sample 10	Sample 10
C	215	215	Sample 11	Sample 11	Sample 12	Sample 12	Sample 13	Sample 13	Sample 14	Sample 14	Sample 15	Sample 15
D	100	100	Sample 16	Sample 16	Sample 17	Sample 17	Sample 18	Sample 18	Sample 19	Sample 19	Sample 20	Sample 20
E	46	46	Sample 21	Sample 21	Sample 22	Sample 22	Sample 23	Sample 23	Sample 24	Sample 24	Sample 25	Sample 25
F	22	22	Sample 26	Sample 26	Sample 27	Sample 27	Sample 28	Sample 28	Sample 29	Sample 29	Sample 30	Sample 30
G	10	10	Sample 31	Sample 31	Sample 32	Sample 32	Sample 33	Sample 33	Sample 34	Sample 34	Sample 35	Sample 35
H	0	0	Sample 36	Sample 36	Sample 37	Sample 37	Sample 38	Sample 38	Sample 39	Sample 39	Sample 40	Sample 40

Duplicate Standard | Duplicate Samples

- Copy the raw plate data from your plate reader software and paste it into the Excel result template under the ‘Raw Data’ sheet.
- Check that the standard curves displayed in the graph are consistent and there are no anomalous points.
- Adjust the dilution factor on the ‘Report’ sheet as appropriate.

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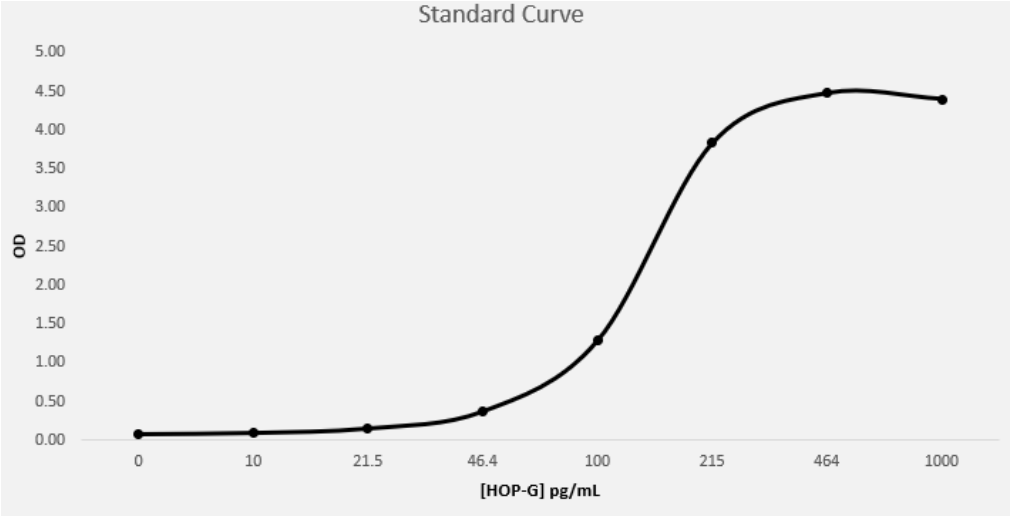
Standard Curve:

The following standard curve should be observed to ensure the assay has been performed correctly.



If the standard curve produced does not resemble the shape above, contact Bioventix for troubleshooting advice.

OD readings outside of the accurate measuring range of the standard curve (22-220 pg/mL) will be reported as ‘Too High’ or ‘Too Low’ accordingly. It is suggested that these samples are repeated at a more appropriate dilution.

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