14,15 DHET ELISA Assay Kit

Catalog Number: 14D39-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 2.2 (26 APR 24)

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences 14,15 DHET ELISA Assay kit is intended for the quantitative determination of 14,15 DHET in biological samples by enzyme linked immunoassay (ELISA). The Eagle. Biosciences 14,15 DHET ELISA Assay kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY BACKGROUND

The level of 14,15-DHET or 14,15-DHET epitope has been shown to exhibit correlation with hypertension in rodents1,2,3 14,15-DHET is a representative metabolite of cytosolic epoxide hydrolase-mediated metabolism of EET's, which are generated by arachidonic acid epoxygenase activity of cytochrome P450's4.

This is a competitive ELISA kit, based on competition between 14,15-DHET epitope and 14,15DHET-HRP conjugate for limited number of binding sites available from the anti-14,15-DHET antibody, which is coated to the wells of the 96 well ELISA plate. The conjugate concentration is held as a constant in each well, while the concentration of the 14,15-DHET is variable, based on the concentration of the sample or standard. Thus the amount of the 14,15-DHET conjugate which is able to bind to each of the wells is inversely proportional to the concentration of 14,15-DHET in the standard or sample. The amount of the conjugate which is bound to each well is then determined by the amount of color obtained, when TMB is added. The TMB reacts with the HRP in the conjugate forming a blue color in the well, which will be more or less intense based upon the amount of HRP available in the well. With the addition of sulfuric acid, the blue colored product is converted to a yellow colored product, which can be read on a plate reader at 450 nm. For additional information see NOTE.

MATERIALS PROVIDED

Item	Description	Quantity
14,15-DHET ELISA Plate	Solid 96-well plate coated with anti14,15-DHET antibody per well	1
14,15-DHET Standard (2µL)	Stock standard at a concentration of 1 mg/mL	1
14,15-DHET HRP Conjugates (12 µL)	1000 X concentrated solution	1
Sample Dilution Stock Buffer (25 mL)	10 X solution of Tris-buffered saline with preservatives	1
HRP Buffer (15 mL)	1 X solution of Tris-buffered saline with preservatives	1

Wash Buffer Stock Solution (25 mL)	10 X solution of Tris-buffered saline with detergents and preservatives	1
TMB Substrate (22 mL)	A solution of TMB (tetra methyl benzadine)	1

MATERIALS REQUIRED BUT NOT PROVIDED

- Plate reader with a 450 nm filter
- An 8-channel adjustable pipettor and an adjustable pipettor
- Storage bottles
- Costar® cluster tubes (1.2 mL) and microcentrifuge tubes
- Deionized water

PRECAUTIONS

- Please read all instructions carefully before beginning the 14,15 DHET ELISA Assay.
- The reagents in this kit have been tested and formulated to perform optimally. This 14,15 DHET ELISA Assay kit may not perform correctly if any of the reagents are replaced or any of the procedures are modified.
- This 14,15 DHET ELISA Assay kit is intended for research use only and is not to be used as a diagnostic

ASSAY PREPARATIONS

Note: Remove all of the reagents required, including the TMB, and allow them to equilibrate to room temperature before proceeding. It is necessary to thoroughly mix the concentrated buffer.

Wash Buffer: Mix the solution, applying low heat until a clear colorless solution is obtained. Dilute the entire contents of the Wash Buffer Concentrate (25 mL) with 225 mL of deionized water to yield a final volume of 250 mL of 1 X Wash Buffer. This can then be refrigerated for the entire life of the kit.

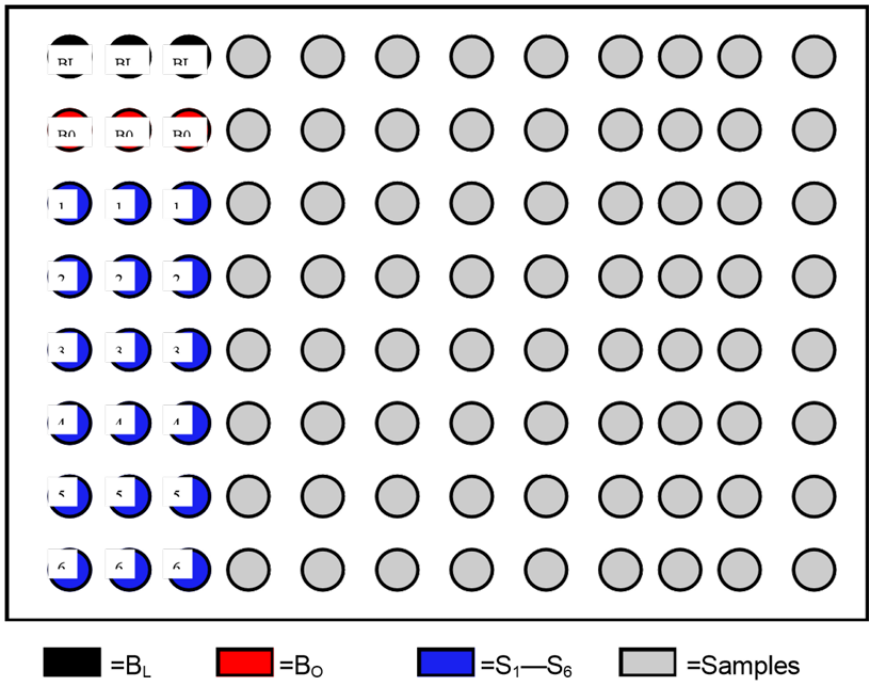
HRP Conjugate: Dilute 1 vial of the 14,15-DHET-HRP conjugate (0.012 mL) with 12.00 mL of 1 X HRP buffer. One vial makes enough conjugate for one plate. The conjugate must be used the same day and should not be stored for later use.

Standards: Label 5 microtubes as Standard 1 through Standard 5. Dilute the entire contents of Sample Dilution Stock buffer (25 mL) with 225 mL deionized water to yield a final volume of 250 mL of 1 X Sample Dilution Buffer. Add 0.9 mL of the Sample Dilution Buffer to the microtubes for Standards 1 to 5. Spin down the enclosed 14,15-DHET standard vial (2 µL, filled with inert gas) and add 1.998 mL of Sample Dilution Buffer to obtain 2 mL of solution. Label this Standard 6. Add 0.1 mL of the Standard 6 to the microtube labeled Standard 5 and mix thoroughly. Next, add 0.1 mL of Standard 5 into the microtube labeled Standard 4 and mix thoroughly. Continue to serially dilute the standards using 1:10 dilutions for the remaining standards.

Samples: Samples can be directly diluted into the 1 X Sample Dilution Buffer if it is in solution. For extracted and dried samples, it is recommended to dissolve the dried-up samples with a minimal amount of ethanol of N, N-dimethyl-formanmide (DMF, 10 µL to 20 µL) and vortex well. Before ELISA assay, add 100 µL of 1 X Sample Dilution Buffer to make the stock sample solution ready for quantification with ELISA. The stock sample solution can be further diluted to a proper range of concentration for ELISA test.

PERFORMING THE ASSAY

Plate Setup: Each plate must contain a minimum of three blank wells (BL), three maximum binding wells (BO), and a six point standard curve (S1-S6). Each sample should be assayed in triplicate. A suggested plate format is shown below:



Standard Dilutions Table

Standards	Final Concentration (pg/mL)	Add Sample Dilution Buffer (mL)	Serial Dilutions Procedure
No. 6	1,000,000	1.998	2µL of stock solution.
No. 5	100,000	0.9	Add 0.1 mL of No. 6
No. 4	10,000	0.9	Add 0.1 mL of No. 5
No. 3	1,000	0.9	Add 0.1 mL of No. 4
No. 2	100	0.9	Add 0.1 mL of No. 3
No. 1	10	0.9	Add 0.1 mL of No. 2

ASSAY PROCEDURE

1. Load 200 microliters of Sample Dilution Buffer into the maximum binding (BO) wells and the blank (BL) wells.
2. Load 100 microliters of each of the standards into the appropriate wells.
3. Load 100 microliters of each of the samples into the appropriate wells.
4. Load 100 microliters of the diluted 14,15-DHET-HRP conjugate in the BO wells, the standard wells, and the sample wells. Do NOT add HRP conjugate into the blank (BL) wells.
5. Incubate the plate at room temperature for two hours.
6. Wash the plate three times with 400 microliters of the diluted Wash Buffer per well.
7. After the last of the three wash cycles pat the plate dry onto some paper toweling.
8. Add 200 microliters of the TMB substrate to all of the wells (including BL wells).
9. Incubate the plate at room temperature for 15-30 minutes.
10. Add 50 microliters of 2 N sulfuric acid to all of the wells.
11. Read the plate at 450 nm.

CALCULATION OF RESULTS

Most plate readers provide data reduction software that can be used to plot the standard curve and determine the sample concentrations. If your plate reader does not have this option, then a data reduction program can be used (4 parameter, of log-log curve fit). If you do not have these options, the results can be obtained manually as follows:

1. Average the absorbance readings from the blanks and subtract that value from each well of the plate to obtain the corrected readings. (Note: Some plate readers do this automatically. Consult the user manual of your plate reader.)

2. Average the corrected absorbance readings from the BO wells. This is your maximum binding.

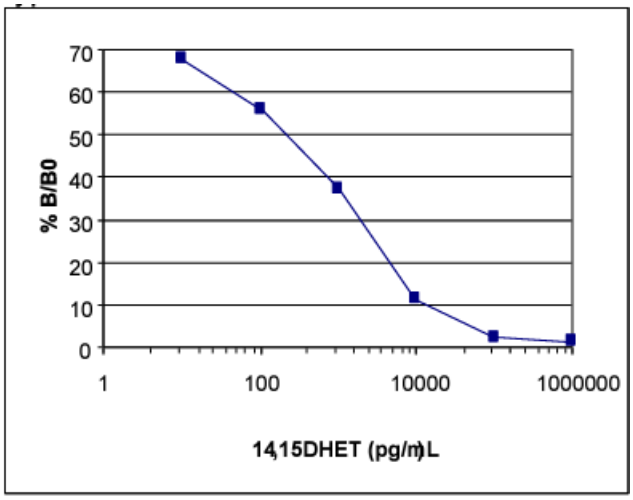
3. Calculate the %B/BO for Standard 1 by averaging the corrected absorbance of the two S1 wells, divide the average by the maximum binding, then multiply by 100. Repeat this formula for the remaining standards.

4. Plot the %B/BO versus the concentration of 14,15-DHET from the standards using semi-log paper.

5. Calculate the %B/BO for the samples and determine the concentrations, utilizing the standard curve.

6. Multiply the concentrations obtained for each of the samples by their corresponding dilution factor.

Typical Results



The data shown here is an example of typical results obtained using the Eagle Biosciences 14,15DHET ELISA kit. These results are only a guideline, and should not be used to determine values from your samples. The user must run their own standard curve every time.

BL wells = 0.070

BO wells = 0.869

Standard	Concentration	O.D.	%B/B_o
No. 1	10 pg/mL	2.330	99.70
No. 2	100 pg/mL	2.200	94.20
No. 3	1,000 pg/mL	1.805	77.3
No. 4	10,000 pg/mL	0.783	33.50
No. 5	100,000 pg/mL	0.256	11.40
No. 6	1,000,000 pg/mL	0.153	6.60

SPECIFICITY OF ANTI-14,15-DHET IgG

Analyte	Specificity
14,15-DHET	100.00%
8,9-DHET	3.30%
11,12-DHET	3.30%
14,15-EET	1.5%
15(s) HETE	100%
8,9-EET	0.40%
5(s)15(s) DiHETE	0.20%

11,12-EET	0.05%
Arachidonic Acid	0.05%
5,6-DHET	0.02%
5,6-EET	0.02%
Thromboxane B_2	0.02%
PGE_2	<0.01%
PGF_{2a}	<0.01%
6-Keto-PGF_{1a}	<0.01%

*Recent experiment showed 0.3% cross-reactivity

Production of 14,15-DHET ELISA

A goat was immunized with 14,15-DHET conjugated to KLH via carboxyl group of the 14,15DHET. Specificity of the 14,15-DHET IgG was assayed with authentic eicosanoids structurally similar to 14,15-DHET

Quantitation of 14,15-EET Level Using 14,15-DHET ELISA

The 14,15-DHET ELISA can be used for measurement of 14,15-DHET levels after chemical oxidation of the 14,15-EET to 14,15-DHET. 14,15-EET+14,15-DHET levels have to be obtained. Then, to obtain 14,15-EET levels, 14,15-DHET levels have to be subtracted from 14,15EET+14,15-DHET levels after measuring 14,15-DHET levels.

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***Please contact us for urine or plasma extraction procedure or human study**

TROUBLESHOOTING

No color present in standard wells

- The HRP conjugate was not added. Redo the assay and add the conjugate at the proper step.
- The HRP conjugate was not incubated for the proper time. Redo the assay and incubate for the proper time.

No color in any wells, including the TA wells.

- The TMB substrate was not added. Add substrate.
- The TMB was not incubated for the proper time. Continue incubation until desires color is reached

The color is faint

- One or all the incubation times were cut short. Redo the assay with the proper incubation times.
- The TMB substrate was not warmed up to room temperature. Redo the assay making sure all reagents are at room temperature.
- The lab is too cold. "Be sure the lab temperature is between 21-27ºC and redo the assay.

The background color is very high

- The TMB substrate has been contaminated. Redo the assay with a fresh bottle of substrate.

Scattered O.D. obtained from the sample

- Redo the assay using an 8-channel pipetman making sure that 8 channels are equal volume while loading.

WARRANTY INFORMATION

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