FABP4 ELISA Protocol

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Abstract

Plasma FABP4 concentrations were analyzed blinded to their preeclampsia status with BioVendor Human AFABP ELISA (Cat. No: RD191036200R, Biovendor, Modrice, Czech Republic) in our study. The manufacturer reports a normal range of 19.58 ± 16.32 ng/mL (mean ± 2 SD) for 35- to 52-year-old women. The limit of detection of the FABP4 ELISA assay is 0.05 ng/ml. The antibodies used in this ELISA are specific for human AFABP according to the manufacturer.

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Materials

✓ BioVendor Human AFABP ELISA Cat. No: RD191036200R by Contributed by users

Protocol

Reconstitute Master Standard and prepare set of standards

Step 1.

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking. The resulting concentration of the human AFABP in the stock solution is 25 ng/ml.
Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	25 ng/ml
200 ml of stock	300 ml	10 ng/ml
250 ml of 10 ng/ml	250 ml	5 ng/ml
250 ml of 5 ng/ml	250 ml	2.5 ng/ml
200 ml of 2.5 ng/ml	300 ml	1 ng/ml
250 ml of 1 ng/ml	250 ml	0.5 ng/ml

Prepare Quality Controls (QCs)

Step 2.

Dilute reconstituted Quality Controls 10x with Dilution Buffer.

Prepare the working Biotin Labelled Antibody solution

Step 3.

Adding 1 part Biotin Labelled Antibody Concentrate (100x) with 99 parts Biotin-Ab Diluent.

Prepare Dilute Wash Solution Concentrate (10x)

Step 4.

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution.

Prepare samples

Step 5.

Dilute plasma samples 10x with Dilution Buffer just prior to the assay

Work sheet

Step 6.

Pipet 100 ul of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 25	QC HIGH	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 10	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 5	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 2.5	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 1	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 0.5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC LOW	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Assay precedure

Step 7.

1. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 100 rpm.
2. Wash the wells 5-times with Wash Solution (0.35 ml per well).
3. Add 100 ul of Biotin Labelled Antibody into each well.
4. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 100 rpm.
5. Wash the wells 5-times with Wash Solution (0.35 ml per well).
6. Add 100 ul of Streptavidin-HRP Conjugate into each well.
7. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 100 rpm.
8. Wash the wells 5-times with Wash Solution (0.35 ml per well).
9. Add 100 ul of Substrate Solution into each well. .
10. Incubate the plate for 10 minutes at room temperature. No shaking
11. Stop the colour development by adding 100 ul of Stop Solution.
12. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm.Subtract readings at 630 nm (550-650 nm)

from the readings at 450 nm.