Human GFAP ELISA

Catalog Number: GFP31-K01

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

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INTENDED USE

This ELISA kit is for quantitative determination of Glial Fibrillary Acidic Protein (GFAP) present in human serum or Cerebrospinal Fluid (CSF).

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.

INTRODUCTION

Glial Fibrillary Acidic Protein (GFAP) is a member of the intermediate filament proteins found in the astroglial cells of the Central Nervous System (CNS). The quantitation of GFAP in serum or CSF levels is recognized as a method in the diagnosis of injury to brain. During the injury to brain or spinal cord, GFAP is released into serum and CSF within a few hours after the injury and shown to be a biomarker for Traumatic Brain Injury (TBI) and retinal stress.

ASSAY PRINCIPLE

The GFAP ELISA test is based on the principle of a solid phase enzyme-linked sandwich immunosorbent assay. The assay system utilizes a specific monoclonal antibody directed against a distinct antigenic determinant on the GFAP molecule and is coated on the microtiter wells for the solid phase immobilization of GFAP. A biotin labeled rabbit anti-GFAP antibody is used as a reporter molecule in a sandwich immunoassay and streptavidin conjugated to Horse radish peroxidase (HRP) is used as the detector molecule. The test sample (serum) is allowed to react with the capture antibody which immobilizes GFAP present in the sample. Following washing, biotinylated polyclonal reporter antibody is added to the wells resulting in the GFAP molecule being sandwiched between the solid phase and biotin-labeled antibodies. After an additional incubation with streptavidin-HRP the wells are washed, a TMB substrate solution is added, and the relative absorbance units (AU) are measured spectrophotometrically using a microtiter plate reader at 450nm. The concentration of GFAP is directly proportional to the AUs of the test sample and is determined from the standard curve.

MATERIALS PROVIDED

Description	Amount
Antibody-Coated Microtiter wells coated with monoclonal anti-GFAP antibody	1 break-apart plate, 96 wells
Calibrator set containing lyophilized GFAP	3 vials (4000 pg/vial)
Sample Diluent	5x (3 mL)
Calibrator/patient serum diluent	12 mL
Biotin-Rb-anti-GFAP reagent	12 mL
Streptavidin-HRP conjugate reagent	12 mL
Wash buffer concentrate	2x (25 mL of 10x PBST)
TMB substrate	12 mL
Stop solution	6 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes: 50 µl, 100 µl, and 1.0 ml
- Disposable pipette tips
- Deionized water
- Vortex mixer or equivalent
- Plate shaker
- Absorbent paper or paper towels
- Microtiter plate reader

STORAGE CONDITONS

- Store the kit at 4-8C upon receipt. Refer to the package label for the expiration date.
- The opened reagents are stable until the expiration date if stored properly at 4-8ºC
- Keep antibody coated microtiter plate dry in the sealed bag with desiccant to minimize exposure to moisture

INSTRUMENT

A microplate reader capable of measuring signals between 450-650 nm.

ASSAY PREPARATIONS

Preparing calibration series:

- In a holder set up seven 1.5 mL tubes such as Eppendorf tubes and label them 2 to 8.
- Add 400 µL of calibrator diluent into each of the 7 tubes.
- Add 1 ml of calibrator diluent in one of the vials containing GFAP calibrator, mix by vortexing to get 4000 pg/ml GFAP solution. Label this vial as #1.
- Make a 2-fold serial dilution of the 4000 pg/ml GFAP solution by transferring 400 µL from tube #1 to #2, #2 to #3 and all the way to #7, to get 2000, 1000, 500, 250, 125 and 62.5 pg/ml GFAP solutions. Note tube #8 has only the calibrator diluent and is the 0 pg/ml GFAP control.

Preparing 1X wash buffer

- Dilute the entire 25 ml of the 10x wash buffer concentrate to 500 ml with distilled water in a bottle and store it capped. The 1x wash buffer is good for 6 months at room temperature.

SPECIMEN COLLECTION AND PREPARATION

- The use of serum samples is required for this test
- Serum specimens from patients should be collected using standard techniques
- Specimens which cannot be assayed within 6 hours after collection may be frozen at-20ºC or lower and will be stable for up to 6 months
- Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing.

- Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µL of 5x Sample Diluent into each well.
3. Dispense 100 µL of GFAP calibrators, test samples and controls into duplicate or triplicate wells (An example of the layout is shown in Figure 1 below).

Well ID	GFAP Standard pg/mL			Samples	
	1	2	3	4	5
A	0	0	0	Sample 1	Sample 1
B	62.5	62.5	62.5	Sample 2	Sample 2
C	125	125	125	Sample 3	Sample 3
D	250	250	250	Sample 4	Sample 4
E	500	500	500	Sample 5	Sample 5
F	1000	1000	1000	Sample 6	Sample 6
G	2000	2000	2000	Sample 7	Sample 7
H	4000	4000	4000	And so on	And so on

4. Thoroughly mix for 20-30 seconds on a plate shaker.
5. Incubate at 37° C (or at room temperature if a 37° C shaker is not available) for 60 minutes on a plate shaker.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. (Alternatively. A plate/strip washer can be used.)
8. Wash the microwells 3-4 times with 300 µL of 1x wash buffer/well.
9. Add 100 µL/well of the Biotin-Rb-anti-GFAP reagent.
10. Incubate at 37° C (or at room temperature if a 37° C shaker is not available) for 60 minutes on a plate shaker.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. (Alternatively, a plate/strip washer can be used.)
13. Wash the microwells 3-4 times with 300 µL of 1x wash buffer/well.
14. Add 100 µL/well of the Streptavidin-HRP conjugate reagent.
15. Incubate at 37° C (or at room temperature if a 37° C shaker is not available) for 30 minutes on a plate shaker.
16. Remove the incubation mixture by flicking plate contents into a waste container.
17. (Alternatively, a plate/strip washer can be used.)
18. Wash the microwells 4-5 times with 300 µL of 1x wash buffer/well.
19. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.
20. Dispense 100 µL of the TMB substrate solution into each well. Gently mix for 5 seconds.
21. Incubate at room temperature for 15 minutes.
22. Dispense 50 µl of the stop solution into each well in the same order as TMB substrate was added.

23. Read the absorbance at 450 nm in each well using a microtiter plate reader.

DATA ANALYSIS

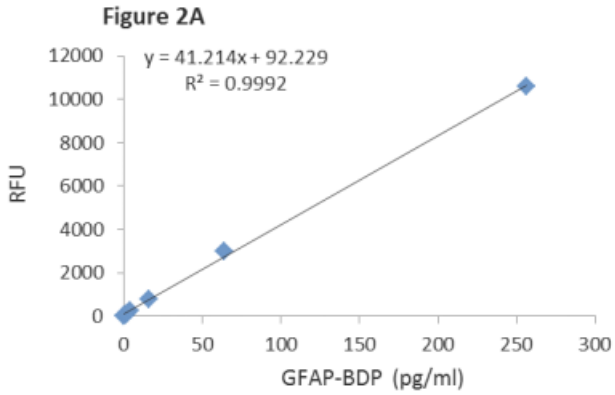
- Calculate the mean luminescence (AU) for each set of reference calibrators, controls and samples.
- Construct a standard curve by plotting the mean AU obtained for each reference calibrator against its concentration in ng/ml, with AU values on the vertical or Y axis, and concentrations on the horizontal or X axis.
- Use the mean AU values for each specimen to determine the corresponding concentration of GFAP in ng/ml from the standard curve.
- Note: Many plate readers come with built-in software for data analysis, which can be used for processing and analyzing the data.

EXAMPLE STANDARD CURVE

- A typical standard curve is shown in Figure 2 below. This standard curve is for illustrative purpose only and should not be used to calculate unknowns. Each laboratory should obtain its own data and standard curve.

GFAP (pg/mL)	AU			Avg AU	Net AU
0	0.095	0.093	0.089	0.092	0.000
62.5	0.122	0.117	0.122	0.120	0.028
125	0.14	0.156	0.166	0.154	0.062
250	0.256	0.223	0.258	0.246	0.153
500	0.379	0.367	0.41	0.385	0.293
1000	0.748	0.642	0.554	0.648	0.556
2000	01.235	1.092	1.178	1.168	1.076
4000	2.199	2.166	2.191	2.185	2.093

Table 1: Typical results form an ELISA showing (triplicate) AU, average AU and net AU (after background subtraction) for each GFAP concentration.



LIMITATIONS AND PRECAUTIONS

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- Do not mix reagents from different kits.
- Do not use previously generated standard curve for data analysis. Generate a fresh standard curve with each assay.
- The wash procedure is critical. Insufficient washing will result in poor precision and false luminescence readings.
- If the AU values exceed the detection limit of the luminometer, the sample must be diluted and retested.

PERFORMANCE AND CHARACTERISTICS

Sensitivity

The assay range for this kit is from 0 to 4000 pg/ml GFAP with a limit of detection (LOD) of <10 pg/ml. If the AU of the sample results in >AU for 4000 pg/ml calibrator, the sample should be diluted and retested.

Precision

Intra-Assay precision was determined by replicate determinations of GFAP at three different concentrations (pg/ml) in serum samples in one assay. Intra-assay variability is shown below:

Sample	4000 pg/mL	1000 pg/mL	250 pg/mL
# Replicates	3	3	3
Mean	3989	1020	267
SD	162	38	15
CV	4%	4%	6%

Inter-Assay precision was determined by replicate determinations of GFAP at three different concentrations (pg/ml) in serum samples in 5 different assays. Inter-assay variability is shown below:

Sample	4000 pg/mL	1000 pg/mL	250 pg/mL
# Replicates	5	5	5
Mean	3936	1040	263
SD	66	105	20
CV	2%	10%	8%

RECOVERY

Serum samples from healthy individuals with GFAP concentration < 10 pg/ml were spiked with known amounts of recombinant GFAP and assayed in triplicate. The mean recovery was ~90%.

Specificity

The assay does not cross react with UCH-L1, another brain biomarker.

Stability

The kit along with all the components is stable for at least six months when stored at 4-8° C. The lyophylized calibrator should be used within 4 hrs after reconstitution.

REFERENCES

1. Engvall, E., "Methods in Enzymology", Volume 70, VanVunakis H. and Langone, J.J. (eds.), Academic Press, New York, NY, 419-492, (1980).

WARRANTY INFORMATION

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