Package Insert

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Human GFAP Chemiluminescence Assay Kit

Catalog Number:
GFP31-L01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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1. Intended Use

The Human GFAP Chemiluminescence Assay Kit ELISA kit is for quantitative determination of Glial Fibrillary Acidic Protein (GFAP) present in human serum or Cerebrospinal Fluid (CSF).
This kit is for research use only and should not be used for patient diagnosis and treatment.

2. Summary

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, plasma 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/plasma and CSF within a few hours after the injury, and shown to be a biomarker for Traumatic Brain Injury (TBI) and retinal stress.

3. Principle of the Assay

The GFAP ELISA test is based on the principle of a solid phase enzyme-linked sandwich immunosorbent assay (1, 2). 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 chemiluminescent substrate solution is added and the relative luminescence units (RLU) are measured spectrophotometrically using a luminometer. The concentration of GFAP is directly proportional to the RLUs of the test sample and is determined from the standard curve.

4. Reagents

Reagents	Quantity
Antibody-Coated Microtiter wells coated with monoclonal anti-GFAP antibody	1 break-apart plate, 96 wells
Calibrator set containing lyophilized GFAP	640 pg/vial, 3 vials/kit
Sample Diluent or Disruption Buffer, 5x	3 ml
Calibrator/patient serum diluent	12 ml of human serum with preservatives
Biotin-Rb-anti-GFAP reagent	12 mL
Streptavidin-HRP conjugate reagent	12 ml
Wash buffer concentrate	25 ml of 20x PBST
Chemiluminescent substrate A and B	6 ml each

5. 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

6. Storage Instructions

- Store the kit at 2-8°C upon receipt. Refer to the package label for the expiration date.
- The opened reagents are stable until the expiration date if stored properly at 2-8°C.
- Keep antibody coated microtiter plate dry in the sealed bag with desiccant to minimize exposure to moisture.

7. Instrumentation

A microtiter luminometer capable of measuring luminescent signal.

8. Specimen Collection, processing, and storage

- 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 -20 ˙C or lower and will be stable for up to six 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.

9. Assay Preparation

9.1 Preparing calibartion series:

- In a holder set up seven 1.5 ml tubes such as eppendorf tubes and label them 2 to 8.
- Add 400 ·L 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 640 pg/ml GFAP solution. Label this vial as #1.
- Make a 2-fold serial dilution of the 640 pg/ml GFAP solution by transferring 400 ·l from tube #1 to #2, #2 to #3 and all the way to #7, to get 320, 160, 80, 40, 20 and 10 pg/ml GFAP solutions. Note tube #8 has only the calibrator diluent and is the 0 pg/ml GFAP control.

9.2 Preparing 1x wash buffer:

Dilute the entire 25 ml of the 20x 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.

10. Performing the Assay

- Secure the desired number of coated wells in the holder.
- Dispense 25 µl of 5x Sample Diluent into each well.
- 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	10	10	10	Sample 2	Sample 2
C	20	20	20	Sample 3	Sample 3
D	40	40	40	Sample 4	Sample 4
E	80	80	80	Sample 5	Sample 5
F	160	160	160	Sample 6	Sample 6
G	320	320	320	Sample 7	Sample 7
H	640	640	640	and so on	

Figure 1: A typical plate layout of the GFAP ELISA

- Thoroughly mix for 20-30 seconds on a plate shaker.
- Incubate at 37° C (or at room temperature if a 37° C shaker is not available) for 60 minutes on a plate shaker.
- Remove the incubation mixture by flicking plate contents into a waste container. (Alternatively. a plate/strip washer can be used.)
- Wash the microwells 3-4 times with 300 µl of 1x wash buffer/well.
- Add 100 µl/well of the Biotin-Rb-anti-GFAP reagent.
- Incubate at 37° C (or at room temperature if a 37°C shaker is not available) for 60 minutes on a plate shaker.
- Remove the incubation mixture by flicking plate contents into a waste container. (Alternatively, a plate/strip washer can be used.)
- Wash the microwells 3-4 times with 300 µl of 1x wash buffer/well.
- Add 100 ·l/well of the Streptavidin-HRP conjugate reagent.
- Incubate at 37°C (or at room temperature if a 37° C shaker is not available) for 30 minutes on a plate shaker.
- Remove the incubation mixture by flicking plate contents into a waste container. (Alternatively, a plate/strip washer can be used.)
- Wash the microwells 4-5 times with 300 µl of 1x wash buffer/well.

- Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.
- While the samples are incubating prepare required volume of the chemiluminescent substrate by mixing equal volume of reagents A and B in a separate tube.
- Dispense 100 µl of the chemiluminescent substrate solution into each well. Gently mix for 5 seconds.
- Incubate at room temperature for 5 minutes.
- Read the luminescence in each well using a luminometer plate reader.

11. Data Analysis

- Calculate the mean luminescence (RLU) for each set of reference calibrators, controls and samples.
- Construct a standard curve by plotting the mean RLU obtained for each reference calibrator against its concentration in ng/ml, with RLU values on the vertical or Y axis, and concentrations on the horizontal or X axis.
- Use the mean RLU 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.

12. Example of 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)	RLU			Avg RLU	Net RLU
0	3061	3224	3025	3103	0
10	3797	3707	3615	3706	603
20	4303	4289	4161	4251	1148
40	6930	6230	5829	6330	3226
80	8132	7836	8052	8007	4903
160	14245	12969	13171	13462	10358
320	23991	22861	22159	23004	19900
640	40989	42302	43257	42183	39079

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

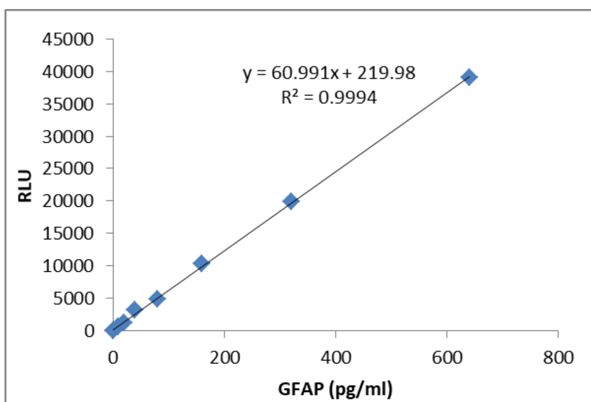

Figure 2: A typical standard curve showing linear fit of the data with R^2 value of 0.9994.

13. Assay 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 RLU values exceed the detection limit of the luminometer, the sample must be diluted and retested.

14. Performance Characteristics

Sensitivity:
The assay range for this kit is from 0 to 640 pg/ml GFAP with a limit of detection (LOD) of <10 pg/ml. The samples containing > 640 pg/ml GFAP (which results in RLU greater than that for 640 pg/ml calibrator) 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	640 pg/ml	160 pg/ml	40 pg/ml
# Replicates	6	6	6
Mean RLU	42901	13439	6041
SD	626	419	263
CV	1%	3%	4%

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	640 pg/ml	160 pg/ml	40 pg/ml
# Replicates	5	5	5
Mean RLU	41331	13020	5679
SD	2373	730	701
CV	6%	6%	12%

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.

15. References

1. Engvall, E., "Methods in Enzymology", Volume 70, VanVunakis H. and Langone, J.J. (eds.), Academic Press, New York, NY, 419-492, (1980).
2. Uotila, M., Ruouslahti, E. And Engvall, E., J. Immunol. Methods, 42, 11-15, (1981).

Warranty Information

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