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GFAP (Human) ELISA Kit

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(Catalog # E5049-100, 96 assays; Store at 4°C)

I. Introduction:

GFAP, Glial Fibrillary Acidic Protein is an intermediate filament protein which belongs to the class-III intermediate filament family. GFAP is expressed in numerous cell types of the central nervous system, ependymal cells and phosphorylated by PKN1. GFAP acts as a cell-specific marker during the development of the central nervous system and distinguishes astrocytes from other glial cells. It is involved in many important CNS processes, including cell communication and the functioning of the blood brain barrier. GFAP and its breakdown products are rapidly released into biofluids during traumatic brain and spinal cord injuries and stroke, making them a strong candidate bio-markers for such neurological disorders. **BioVision's GFAP (Human) ELISA Kit** is used for the quantitative detection of GFAP in serum, plasma, tissue homogenates and other biological fluids. It is based on the principle of sandwich ELISA. The capture antibody is pre-coated on 96-well plates. The standards, test samples and biotin conjugated detection antibody are added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin is added and unbound conjugates are washed away with wash buffer. The HRP enzymatic reaction is detected using TMB-substrate. Finally, an acidic stop solution terminates the enzymatic reaction. The color developed is directly proportional to the amount of GFAP in the sample.

II. Features and Benefits:

- Detection range: 0.313-20 ng/ml
- Sensitivity: 0.188 ng/ml
- Assay Precision; Intra-Assay CV < 8% and Inter-Assay < 10%
- Recovery Rate: 85-104% for Serum, 87-103% for EDTA plasma and 87-99% for Heparin plasma
- This sandwich ELISA is highly sensitive and specific for the detection of GFAP. There is no significant cross-reactivity or interference between GFAP and analogues.

III. Sample Types:

Human Serum, Plasma, Tissue lysates and other biological fluids

IV. Kit Contents:

Components	E5049-100	Storage Temperature	Part Number
ELISA Microplate	8x12 Strips	4 °C	E5049-100-1
Lyophilized Standard (20 ng)	2 vials	4 °C	E5049-100-2
Sample/Standard Dilution Buffer	20 ml	4 °C	E5049-100-3
Biotin-labeled Antibody (Concentrated)	120 µl	4 °C (Avoid Light)	E5049-100-4
Antibody Dilution Buffer	10 ml	4 °C	E5049-100-5
HRP-Streptavidin Conjugate (SABC)	120 µl	4 °C (Avoid Light)	E5049-100-6
SABC Dilution Buffer	10 ml	4 °C	E5049-100-7
TMB Substrate	10 ml	4 °C (Avoid Light)	E5049-100-8
Stop Solution	10 ml	4 °C	E5049-100-9
Wash Buffer (25X)	30 ml	4 °C	E5049-100-10
Plate Sealers	5	4 °C	E5049-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Shipping and Storage Conditions:

The entire kit can be shipped at 4°C. The kit should be stored at 4°C for up to 6 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Bring all reagents and samples to room temperature (RT) for 20 min before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.
After opening and before using, keep plate dry.
Don't let microplate dry during the assay, as dry plate will inactivate active components on plate.

Wash Buffer: Dilute 25X Wash Buffer to 1X by adding 30 ml of 25X Wash Buffer and make up the volume to 750 ml with deionized/distilled water. If crystals are present in the 25X Wash Buffer, warm it in a water bath at 40°C. Mix it gently. The solution must be cooled to RT before use. Store unused solution at 4°C.

1. Standard Preparation:

- Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as standard tube). Keep the tube at RT for 10 min. Mix thoroughly.
- Label 7 tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the Sample Dilution Buffer into each tube. Add 0.3 ml of the above Standard solution (from standard tube) into 1st tube and mix thoroughly.
- Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them

thoroughly, and so on. Sample Dilution Buffer iss used for the blank control. (Note: Please use Standard Solutions within 2 h of preparation).

Note: If the standard tube concentration is higher than the range of the kit, please dilute and use.

2. **Biotin-labeled Antibody working solution**: Prepare this working stock 1 h prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 ml/well x quantity of wells. Add 0.1-0.2 ml to the total volume. Dilute the Biotin-labeled antibody with Antibody Dilution Buffer at 1:100. Mix thoroughly.
3. **HRP-Streptavidin Conjugate (SABC)**: Prepare this working stock 30 min prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 ml/well x quantity of wells. Add 0.1-0.2 ml to the total volume. Dilute the SABC with SABC Dilution Buffer at 1:100. Mix thoroughly.
4. **Sample Preparation:**

Note: Isolate the test samples soon after collecting, then, analyze immediately. Alternatively, aliquot and store at -20 °C (≤ 1 month) or -80 °C (≤ 2 months). Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this assay

- **Serum:** Place whole blood sample at RT for 2 h or keep at 4 °C overnight and centrifuge for 20 min at approximately 1000xg. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin free.
- **Plasma:** Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000xg at 4°C within 30 min of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates**: As hemolytic blood may affect the assay result, it is necessary to remove residual blood by washing tissue with pre-cooled PBS buffer (0.01 M, pH 7.4). Mince tissue after weighing it and homogenize it in PBS (the volume depends on the weight of the tissue). Normally, 9 ml PBS would be appropriate for 1 gram tissue pieces. Some protease inhibitors are recommended to be added to the PBS. Homogenize using a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/ml.
- **Cell culture supernatant:** Centrifuge supernatant for 20 min at 1000xg at 4 °C to remove insoluble impurities and cell debris. Collect the clear supernatant and carry out the assay immediately.
- **Cell Culture Lysate:** Commercial RIPA kits are recommended. Follow the instructions provided. Generally, 0.5 ml RIPA lysis buffer would be appropriate for $2x10^6$ cells, DNA must be removed. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/ml.
- **Other Biological fluids:** Centrifuge samples for 20 min at 1000xg at 4°C. Collect the supernatant and carry out the assay immediately.

Note: End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. **The matrix components in the sample may affect the test results. Please dilute the sample ½ with Sample Dilution Buffer before testing.**

VIII. Assay Protocol:

Note: When diluting samples and reagents, the solutions must be mixed completely and evenly.

Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

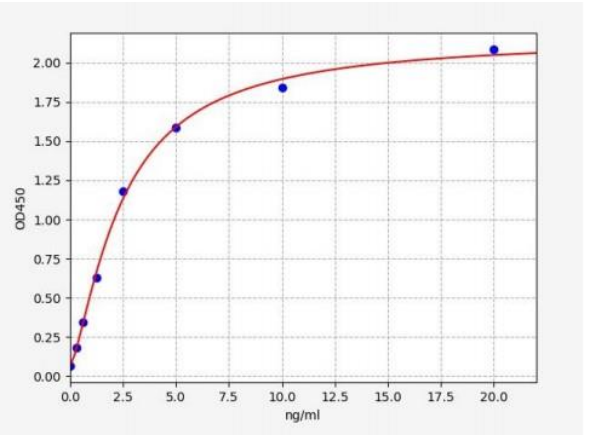
1. Prepare all reagents, **samples** (diluted ½ with Sample Dilution Buffer) and standards as instructed in section VII.
2. Wash plate 2 times with **1X Wash Buffer** before adding standard, sample (diluted ½ with Sample Dilution Buffer) and control (blank) wells.
3. Add 100 µl of each **standards or samples** into appropriate wells. Seal the plate with a cover and incubate for 1.5 hours at 37 ºC.
4. Remove the cover and discard the plate content. Wash the plate 2 times with 1X Wash Buffer without letting the wells get completely dry.
5. Add 100 µl of **Biotin-labelled antibody** work solution into the above wells. Seal the plate and incubate at 37 ºC for 60 min.
6. Discard the solution and wash 3 times with **1X Wash Buffer**. Wash by filling each well with Wash Buffer (350 µl) using a multichannel pipette or autowasher. Let it soak for 1-2 min, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Tap the plate on absorbent filter papers or other absorbent materials.

   Note: Washing process is very important. If washing is not done well, it may cause a false positive and high background.
7. Add 100 µl of **SABC working solution** into each well, cover the plate and incubate at 37 ºC for 30 min.
8. Discard the solution and wash 5 times with **1X Wash Buffer** as described in step 6.
9. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 10-20 min. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. The reaction can be terminated when apparent gradient appears in standard wells).
10. Add 50 µl of **Stop Solution** to each well. Read the absorbance at 450 nm within 20 min using a Microplate Reader.

IX. Calculation:

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) Vs the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. Professional software can also be used for the calculations. If the samples measured are diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Figure: Typical Standard Curve and OD values are shown below: These standard curves are for demonstration only.



STD (ng/ml)	OD-1	OD-2	Average	Corrected
0	0.065	0.067	0.066	0.000
0.312	0.18	0.186	0.183	0.117
0.625	0.337	0.347	0.342	0.276
1.25	0.619	0.637	0.628	0.562
2.5	1.161	1.195	1.178	1.112
5	1.564	1.61	1.587	1.521
10	1.815	1.867	1.841	1.775
20	2.056	2.116	2.086	2.02

X. Recovery

Matrices mentioned below were spiked with certain level of GFAP and the recovery rates were calculated by comparing the measured value to the expected amount of GFAP in samples.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	85-104	94
EDTA Plasma (n=5)	87-103	98
Heparin Plasma (n=5)	87-99	93

XI. Linearity

Linearity of the assay kit was determined by spiking samples with appropriate concentration of GFAP and their serial dilutions. The results are represented as percentage of calculated concentration to the expected value.

Sample	1:2	1:4	1:8
Serum (n=5)	93-102%	87-101%	85-97%
EDTA Plasma (n=5)	84-101%	83-100%	90-100%
Heparin Plasma (n=5)	82-95%	83-94%	80-99%

XII. Related Products:

Nestin (Human) ELISA Kit (Cat. No. K4191)
Desmin (Human) ELISA Kit (Cat. No. K4190)
IFN-gamma (Human) ELISA Kit (Cat. No. E4825)
GDNF (Human) ELISA Kit (Cat. No. K4184)
HSPB1 (Human) ELISA Kit (Cat. No. E4984)