

Human GFAP ELISA

For the quantitative determination of human GFAP concentrations in cell culture supernatants, serum, and plasma.

INTRODUCTION

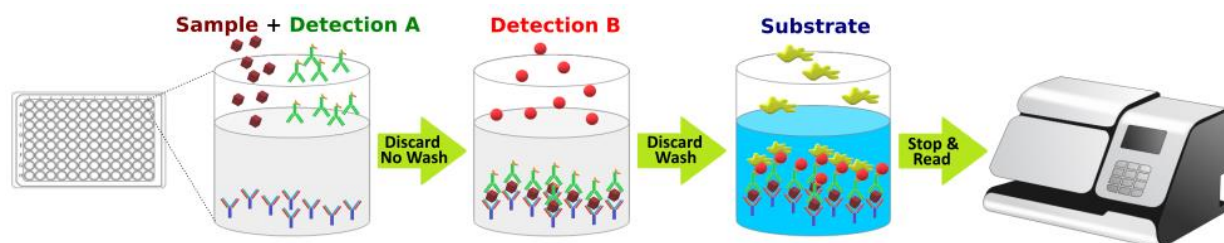
Glial Fibrillary Acidic Protein (GFAP) is a type III intermediate filament (IF) majorly expressed by astrocytes in the central nervous system (CNS). GFAP is a marker to distinguish astrocytes from other glial cells during development. Astrocytes play a variety of key roles in supporting, guiding, nurturing, and signaling neuronal architecture and activity. GFAP is involved in the structure and function of the cell’s cytoskeleton. GFAP is also involved in many important CNS processes, including cell communication and the functioning of the blood brain barrier. As a potential biomarker, GFAP has been shown to associate with multiple diseases such as traumatic brain injury, stroke, brain tumors, etc. Mutations in this gene cause Alexander disease, a rare disorder of astrocytes in the central nervous system. Decreases in GFAP expression have been reported in Down’s syndrome, schizophrenia, bipolar disorder, and depression.

Tribioscience’s Human GFAP ELISA is designed to quantitatively detect human GFAP levels in serum, plasma, and other biological samples. The main feature is that **the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate, and fast (Fig. 1). The detection range is from 4 to 3000 pg/mL.** The levels of human GFAP samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural human GFAP protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific for human GFAP is pre-coated onto a microplate. Standards or samples and a biotin conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well without washing and directly add Streptavidin-HRP into the complex. Following a wash, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is proportional to the amount of GFAP bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

Fig. 1



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human GFAP Microplate	TBS32106A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human GFAP.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human GFAP Standard	TBS32106B	100 µl of Recombinant human GFAP (30 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS32106C	2.1 mL of biotin-human GFAP antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS32106D	200 µL of streptavidin HRP.	
Assay Diluent	TBS32106E	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x)	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date. The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

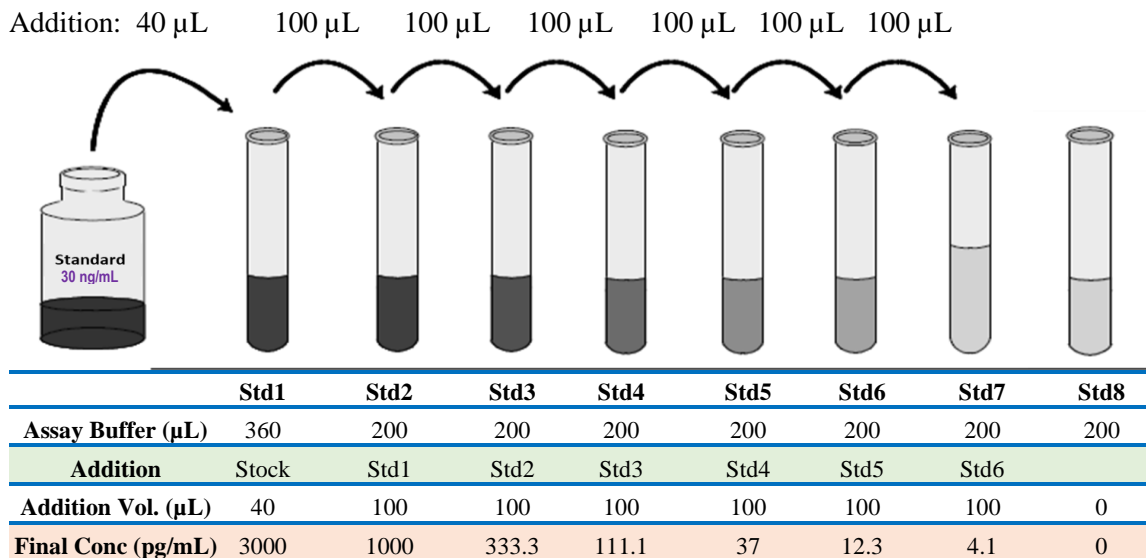
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved*).

Detection B working solution preparation: Add 150 µL of **Detection B** streptavidin-HRP to 12 mL Assay Diluent (TBS32106E) to prepare **Detection B** working solution.

Human GFAP Standard Preparation: Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below**.

1. Add 40 µL of the Human GFAP Standard stock solution (30 ng/mL) to tube #1 and mix.
2. Make 3x serial dilutions of the standard using the Tube#1(3000 pg/mL standard solution) from Tube #2 through #7 with sequential transfer of 100 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 3000, 1000, 333, 111, 37, 12.3 and 4.1 pg/mL. Tube# 8 is Standard 8 (0 pg/mL).

Fig.2 Diagram for Human GFAP standard preparation



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **4°C overnight**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B working solution** to each well. Incubate at **RT for 1 hour with shaking**.
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20 minutes with shaking** (*Protect from light*). The color becomes blue.
7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).

- Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 542 nm or 570 nm. If wavelength correction is not available, subtract readings at 542 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve ($R^2=1.000$) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of human is typically 4 pg/mL.

The Intra-assay CV and the Inter-assay CV are <10%.

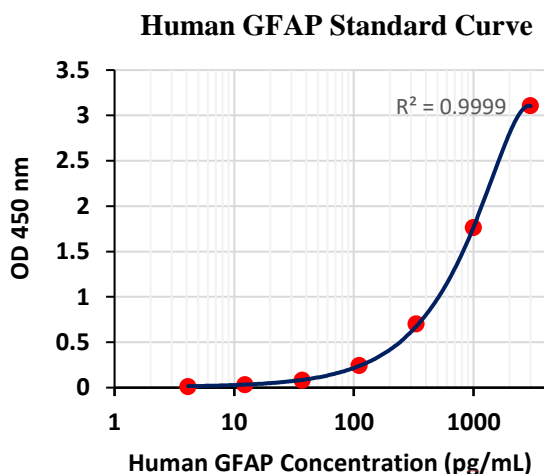
SPECIFICITY

This assay recognizes natural and recombinant human GFAP.

No cross-reactivity with others.

RELATIVE PRODUCTS

- Human p-Tau-217 ELISA (TBS3293)
- Human p-Tau-181 ELISA (TBS3294)
- Human Total Tau ELISA (TBS3295)
- Human p-Tau-231 ELISA (TBS3296)
- Human AD7 Human AD7C NTP (TBS3297)
- Human Amyloid β 40 ELISA (TBS3298)
- Human NF-L ELISA (TBS32101)
- Human Total Amyloid β ELISA (TBS32104)
- Human UCHL1/PGP9.5 ELISA (TBS32107)
- Human Gamma H2AX ELISA (TBS3265)
- Human H2AX ELISA (TBS3266)
- Human IL-4 ELISA (TBS3221)
- Human IL-4 ELISA (TBS3221)
- Human IL-6 ELISA (TBS3223)
- Human IL-7 ELISA (TBS3224)
- Human IL-8 ELISA (TBS3225)
- Human IL-10 ELISA (TBS3226)
- Human IL-13 ELISA (TBS3227)
- Human IL-17 ELISA (TBS3228)
- Human IL-22 ELISA (TBS3229)
- Human IL-33 ELISA (TBS4245)
- Human IFN-gamma ELISA (TBS3230)
- Human TGF- β 1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1 α ELISA (TBS3234)
- Protein Cell Lysis Buffer (TBS5001)



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