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HUMAN GFAP

ENZYME IMMUNOASSAY KIT

catalogue # A05188

96 wells

TABLE OF CONTENTS

Presentation	1
Precautions for use	1
Principle of the assay	1
Materials and equipment required	2
Sample preparation	3
General precautions	3
Sample preparation	3
Reagent preparation	3
Assay procedure	4
Distribution of reagents and samples	4
Pipetting the reagents	4
Incubating the plate	4
Developing and reading the plate	5
Data analysis	6
Typical data	6
Example data	6
Acceptable range	6
Human GFAP standard curve	6
Assay validation and characteristics	7
Assay trouble shooting	8
Bibliography	8

For research laboratory use only.
Not for diagnostic use.

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HUMAN GFAP EIA KIT

96 wells
Storage: 2-8°C
Expiry date: stated on the package

This kit contains:

- ☞A covered 96 well plate, pre-coated with a polyclonal anti-GFAP antibody, ready to use
- ☞One vial of biotin labelled Anti-GFAP antibody, ready to use
- ☞One vial of Streptavidin-HRP tracer, ready to use
- ☞One vial of human GFAP standard, lyophilised
- ☞Two vials of Quality Control: high and low, lyophilised
- ☞One vial of Standard buffer, ready to use
- ☞One vial of Stop solution (0.2 M H_2SO_4), ready to use
- ☞One vial of Substrate solution (TMB), ready to use
- ☞Two vials of EIA buffer, ready to use
- ☞One vial of concentrated Wash buffer (10x), liquid
- ☞One instruction booklet
- ☞One template sheet
- ☞Three well cover sheets

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 40 samples in duplicate.

PRECAUTIONS FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample of reagent and dispense into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.
Not for diagnostic use.
Do not pipet liquids by mouth.
Do not use kit components beyond the expiration date.
Do not mix different lot numbers.
Do not eat, drink, or smoke in area in which kit reagents are handled.
Avoid splashing.

This kit contains components of human origin. These materials were found non-reactive for HbsAg, HCV antibody and for HIV 1/2 antibody and antigen. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents. Wear gloves and laboratory coats are recommended when handling immunodiagnostics materials and samples of human origin.

Stop solution and Substrate solution are potential harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these reagents.

PRINCIPLE OF THE ASSAY

Glial Fibrillary Acidic Protein (GFAP), is the principal 8-9 nm intermediate filament in mature astrocytes of the Central Nervous System (CNS). The findings showed that GFAP is released after traumatic brain injury severity and outcome into the blood very soon after traumatic brain injury (TBI), and that GFAP is not released after multiple trauma without brain injury. In the CNS following injury, either as a result of trauma, disease, genetic disorders, or chemical insult, astrocytes become reactive and insult respond in astrogliosis. Astrogliosis is characterized by rapid synthesis of GFAP. GFAP normally increases with age and there is a wide variation in the collection and processing of human brain tissue. GFAP is also an established biomarker of retinal stress.

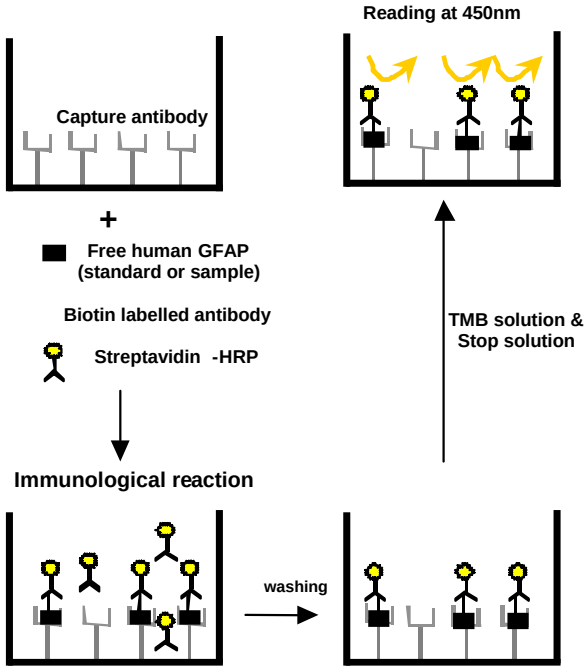
This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a polyclonal antibody specific of human GFAP. This antibody will bind any GFAP introduced in the wells (sample or standard).

After one-hour incubation and a washing, biotin-labelled monoclonal anti-human GFAP antibody is added and incubated with captured GFAP. This allows the two antibodies to form a sandwich by binding on different parts of the human GFAP molecule.

After a thorough wash, streptavidin-horseradish peroxidase tracer is added. The plate is washed to remove any unbounded reagent, and hydrogen peroxide/TMB substrate is added to the wells. The HRP tracer acts on the hydrogen peroxide/TMB substrate to form a yellow compound that absorbs at 450 nm. The reaction is stopped by addition of sulphuric acid solution.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of tracer bound to the well.

The principle of the assay is summarised below:



MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

FOR THE ASSAY

- Precision micropipettes (10 to 1000 µL)
- Spectrophotometer plate reader (450 nm +/- 10 nm filter)
- Microtitration washer (or washbottles)
- Microplate shaker
- Multichannel pipette 100 µL and disposable tips
- Distilled or deionised water
- Polypropylene tubes

SAMPLE PREPARATION

This assay may be used to measure human GFAP in human samples such as serum, plasma, culture supernatant and cerebrospinal fluid.

GENERAL PRECAUTIONS

☞All samples must be free of organic solvents prior to assay.

☞Samples should be assayed immediately after collection or should be stored at -20°C, or preferably at –70°C for long term storage. Avoid repeated freeze/thaw cycles. Mix thoroughly thawed samples just prior to the assay.

☞Avoid using hemolyzed or lipemic samples.

SAMPLE PREPARATION

No prior extraction procedure is necessary.

To measure human GFAP, dilute serum or plasma samples 1/3 in EIA buffer (i.e. 100 µL sample + 200 µL EIA buffer for duplicates). Mix well (not to foam). Vortex is recommended. Do not store the diluted samples.

REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except Standard, Quality Control and wash buffer.

☞Human GFAP standard

Reconstitute GFAP standard with X µL of Standard buffer. The volume X is indicated on the vial of the corresponding standard. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam) at room temperature. The concentration of the human GFAP in the stock solution (S1) is 25 ng/mL.

Volume of Standards	Added volume of Standard buffer	Concentration of reconstituted standards
300 µL of S1	450 µL	S2 (10 ng/mL)
300 µL of S2	300 µL	S3 (5 ng/mL)
300 µL of S3	300 µL	S4 (2.5 ng/mL)
300 µL of S4	450 µL	S5 (1 ng/mL)
300 µL of S5	300 µL	S6 (0.5 ng/mL)
300 µL of S6	300 µL	S7 (0.25 ng/mL)

Then prepare standards as follows:

Dilute reconstituted standards 1/3 in Standard buffer prior to use (i.e. 100 µL standard + 200 µL Standard buffer).

The reconstituted and undiluted standards could be frozen at -20°C for 3 months.

Do not store the diluted (1/3) standards.

☞Quality Controls

Reconstitute Quality Control with X µL of distilled or deionised water. The volume X is indicated on the vial of the corresponding Quality Control. Reconstituted Quality Control is stable until the expiration date (see label on the box) when stored at -20°C.

Dilute the reconstituted Quality Control 1/3 in EIA buffer prior to use (i.e. 100 µL Quality Control + 200 µL EIA buffer). Do not store the diluted (1:3) Quality Control.

☞Wash buffer

Dilute 100 mL of concentrated Wash buffer to 1000 mL with distilled or deionised water.

The diluted wash buffer is stable for one month when stored at 2-8°C.

☞Hydrogen peroxide/TMB solution

Substrate solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

☞Stop solution

Stop solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop solution. Wells that are green in colour indicate that the Stop solution has not mixed thoroughly with the Substrate solution.

ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested on this page. The content of each well may be recorded on the sheet provided with the kit.

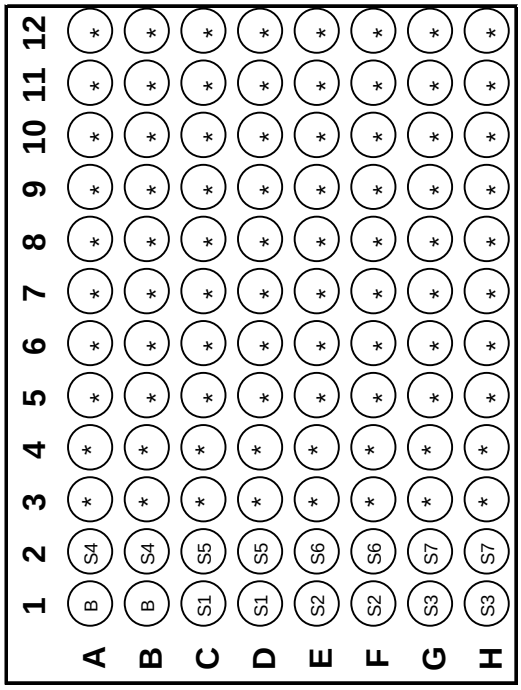
PIPETING THE REAGENTS

All samples and reagents must reach room temperature prior performing the assay. Use different tips to pipet the buffer, standard, sample, tracer antiserum and other reagents.

- Human GFAP standard:
  Dispense 100 µL of each of the seven diluted standards (S1 to S7) in duplicate to appropriate wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.

- Quality Control and samples:
  Dispense 100 µL of diluted Quality Controls and samples to appropriate wells.

- Standard buffer:
  Dispense in duplicate 100 µL to Blank (B) well.



B: Blank
S1-S7: Standards 1-7
*: Samples or Quality Controls

INCUBATING THE PLATE

- Cover the plate with the cover sheet and incubate at room temperature for 2 hours, shaking at 300 rpm on an orbital microplate shaker.

- Rinse each well 3 times with the wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.
- Biotin labelled anti-GFAP antibody
  Dispense 100 µL to each well.
- Cover the plate with the cover sheet and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- Rinse each well 3 times with the wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.
- Streptavidin-HRP tracer:
  Dispense 100 µL to each well.
- Cover the plate with the cover sheet and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- Rinse each well 3 times with the wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.

DEVELOPING AND READING THE PLATE

- Dispense 200 µL of Substrate solution to the 96 wells. Incubate the plate in the dark for 10 to 15 minutes at room temperature. Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil.
- Stop the colour development by adding 50 µL of Stop solution.
- Read the absorbance at 450 nm within 5 minutes following stop solution addition.

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine GFAP concentration of off-scale samples. ***The readings at 405 nm should not replace the on-scale readings at 450 nm***

Enzyme Immunoassay Protocol (Volumes are in µL)			
	Blank	Standard	Sample
EIA Buffer	100		
Standard	-	100	-
Sample	-	-	100
Incubate the plate at room temperature for 2 hours			
Wash the plate 3 times			
Biotin Labelled GFAP	-	100	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 3 times			
Streptavidin-HRP	100	100	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 3 times			
TMB solution	100	100	100
Incubate the plate in the dark at room temperature during 10-15 minutes			
Stop solution	100	100	100
Read the plate at 450 nm			

DATA ANALYSIS

Make sure that your plate reader has substracted the absorbance readings of the blank well (absorbance of TMB solution) from the absorbance readings of the rest of the plate. If not, do it now.

- Using a logit-log graph paper, plot the absorbance for each standard (y axis) versus concentration (x axis) of standards. Draw a best-fit line through the points.

- To determine the concentration of your samples, find the absorbance value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample. Samples with a concentration greater than 25 ng/mL should be re-assayed after dilution.

- Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

TYPICAL DATA

EXAMPLE DATA

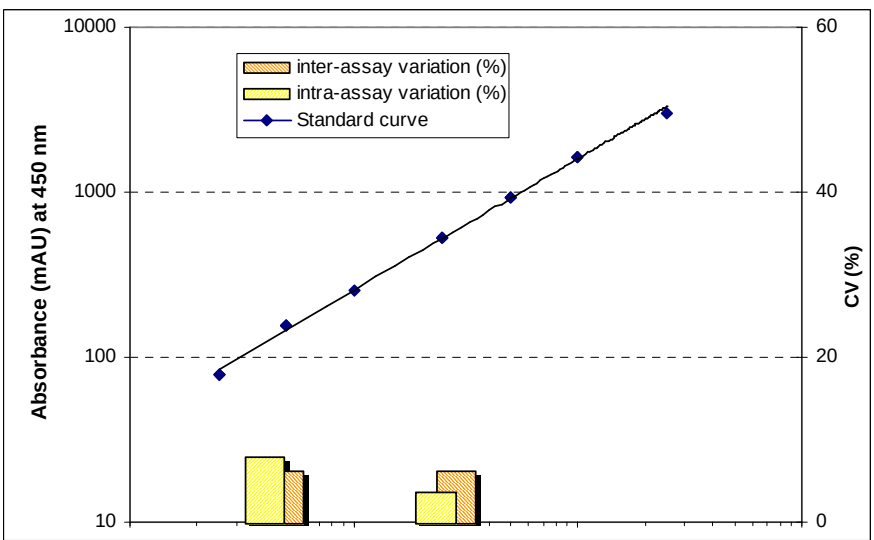
The following data are for demonstration purposes only. Your data may be different but still correct. These data were obtained using all reagents supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Human GFAP	mAU
Blank	29
Standard 25 ng/mL	2 997
Standard 10 ng/mL	1 648
Standard 5 ng/mL	934
Standard 2 ng/mL	530
Standard 1 ng/mL	256
Standard 0.5 ng/mL	156
Standard 0.25 ng/mL	78
QC High	1 148
QC Low	430

ACCEPTABLE RANGE

☞QC samples: see label on the vials.

HUMAN GFAP STANDARD CURVE



ASSAY VALIDATION AND CHARACTERISTICS

The Enzyme Immunometric assay of human GFAP has been validated for its use in human serum, plasma culture supernatant and cerebrospinal fluid.

☞Cross-reactivity :

The assay recognizes natural human GFAP.

- Mouse GFAP < 0.1%
- Goat GFAP < 0.1%
- Rabbit GFAP < 0.1%
- Hamster GFAP < 0.1%
- Horse GFAP < 0.1%

☞Sensitivity:

The limit of detection (defined as such a concentration of GFAP giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3*SD_{blank}$) is better than 0.045 ng/mL of sample.

The EIA buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

☞Precision:

- Intra-assay (n=8)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	2.27	0.08	3.8
2	0.43	0.03	6.4

- Inter-assay (Run-to-Run, n=7)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	2.62	0.20	6.1
2	0.40	0.02	5.2

☞Recovery test:

Sample	**O**bserved (ng/mL)	**E**xpected (ng/mL)	Recovery **O/E** (%)
1	2.04	-	-
	7.83	7.04	111.2
	5.10	5.04	101.2
	2.56	2.54	100.8
2	0.41	-	-
	6.11	5.41	112.9
	1.62	1.41	114.9
	1.01	0.91	111.0
3	0	-	-
	5.20	5.00	104.0
	0.85	1.00	85.0
	0.42	0.50	84.0

☞Dilution test:

Sample	Dilution	**O**bserved (ng/mL)	**E**xpected (ng/mL)	Recovery **O**/**E** (%)
1	-	23.54	-	-
	2x	13.00	11.77	110.5
	4x	6.66	5.89	113.1
	8x	3.34	2.94	113.6
2	-	8.59	-	-
	2x	4.16	4.30	96.7
	4x	2.25	2.15	104.7
	8x	1.10	1.07	102.8
3	-	5.34	-	-
	2x	2.33	2.67	87.3
	4x	1.22	1.34	91.1
	8x	0.57	0.67	85.1

ASSAY TROUBLE SHOOTING

☞Absorbance values too low:
- One reagent has not been dispensed
- Incorrect preparation or reagent storage
- Assay performed before reagents reach room temperature
- Incorrect wavelength when reading the absorbance

☞High signal and background in all wells:
- Inefficient washing
- Overdeveloping; incubation time should be reduced before adding Stop Solution
- Incubation temperature over 30°C

☞High dispersion of duplicates:
- Poor pipetting technique or irregular plate washing.
- Inefficient mixing Standards, Quality Controls or samples

These are a few examples of problems that may occur. If you need further assistance, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).
SPI-BIO offers a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39 30 62 60).

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