GFAP (human)

For laboratory research only. Not for human or veterinary diagnostic use.

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GFAP (human)
Enzyme Immunoassay kit
#A05188.96 wells

For research laboratory use only
Not for human diagnostic use

Fabriqué en France
Made in France

#A11188
Version: 0119

Table of contents

96 wells

Storage: +4°C

Expiry date: stated on the package

This kit contains:

Designation	Item #	Quantity per kit	Form
GFAP precoated 96-well Strip Plate	A08188	1	Ready to use
Streptavidin HRP Tracer	A22010	13 mL	Ready to use
GFAP (human) Biotin-labelled Antibody	A40188	13 mL	Ready to use
GFAP (human) Standard	A06188	1 vial	Lyophilised
GFAP (human) Quality Control High	A10188-H	1 vial	Lyophilised
GFAP (human) Quality Control Low	A10188-L	1 vial	Lyophilised
Standard Buffer	A07015	9 mL	Ready to use
ELISA Buffer	A07010	13 mL	Ready to use
Wash Buffer concentrated 10x	A17012	100 mL	concentrated
HRP Substrate Solution (TMB)	A09010	13 mL	Ready to use
Stop Solution	A22000	13 mL	Ready to use
Technical Booklet	A11188	1	-
Well cover Sheet	-	3	-

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.

Precaution for use

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

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- 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 harmful solutions. To avoid any contact, wear eye, hand, face and clothing protection when handling these.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Background

Glial Fibrillary Acidic Protein (GFAP), is the principal 8-9 nm intermediate filament in mature astrocytes of the Central Nervous System (CNS). GFAP is highly brain specific protein that is not found outside the CNS. Some studies showed that GFAP is released into the blood very soon after traumatic brain injury, that GFAP is related to brain injury severity and outcome 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 respond in a typical manner, termed astrogliosis. Astrogliosis is characterized by rapid synthesis of GFAP. GFAP normally increases with age and there is a wide variation in collection and processing of human brain tissue. Thanks to the high brain specificity and early

releasing from CNS after traumatic brain injury, GFAP might be suitable marker for early diagnostics.

Principle of the assay

This Enzyme Immunometric Assay (EIA/ELISA) is based on a sandwich technique. The wells of the plate supplied are coated with a polyclonal antibody specific to Human GFAP.

Human GFAP introduced into the wells (standard or sample) will be bound by the polyclonal antibody coated on the plate and is then detected by a monoclonal antibody tagged with biotin also specific for Human GFAP.

The two antibodies then form a sandwich by binding on different parts of the Human GFAP.

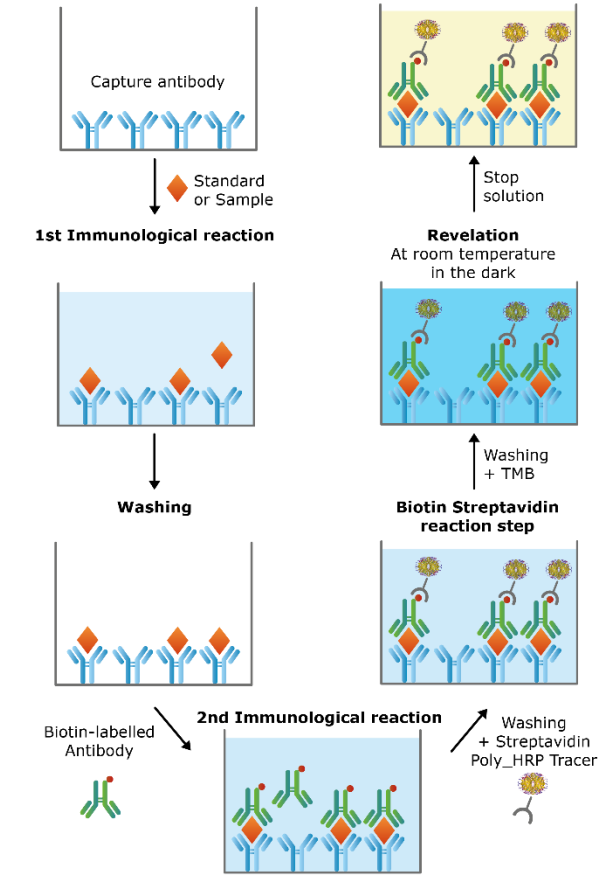
The sandwich is immobilised on the plate so reagents in excess may be washed away.

The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP (Tracer).

The concentration of Human GFAP is determined by measuring the enzymatic activity of immobilized Tracer using TMB. The Tracer acts on TMB to form a yellow compound after the reaction has been stopped.

The intensity of the colour, which is determined by spectrophotometry at 450 nm, is proportional to the amount of Human GFAP present in the well during the immunological incubation.

The principle of the assay is summarised below:



Assay characteristics

- ***Validated for use with human serum, plasma, culture supernatant and cerebrospinal fluid.***
- ***Limit of detection (LOD):*** 0.045 ng/mL (calculated as a concentration of GFAP giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank, with ELISA buffer pipetted into blank wells)
- ***Cross-reactivity***

Molecule/Species	Cross-reactivity
Mouse GFAP	<0.1%
Goat GFAP	<0.1%
Rabbit GFAP	<0.1%
Hamster GFAP	<0.1%
Horse GFAP	<0.1%

- ***Assay validation data:*** ask Bertin Bioreagent (tech@bertin-bioreagent.com) or your local distributor for a copy of the validation data.

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 filter), preferably with reference wavelength 630 nm
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300µL
- 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 from 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 samples 1:3 in ELISA buffer (i.e. 100 µL sample + 200 µL ELISA 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 (around +20°C) 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.

Then prepare standards as follows:

Standard	Volume of Standard	Added volume of Standard buffer	Concentration of reconstituted standards
S1	-	-	25 ng/mL
S2	300 µL of S1	450 µL	10 ng/mL
S3	300 µL of S2	300 µL	5 ng/mL
S4	300 µL of S3	300 µL	2.5 ng/mL
S5	300 µL of S4	450 µL	1 ng/mL
S6	300 µL of S5	300 µL	0.5 ng/mL
S7	300 µL of S6	300 µL	0.25 ng/mL

Dilute reconstituted standards (25 – 0.25 ng/mL) to 1:3 in Standard buffer just prior to use (i.e. 100 µL standard + 200 µL Standard buffer).

The reconstituted and undiluted standards (25 – 0.25 ng/mL) could be aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

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

GFAP Quality Controls

Reconstitute Quality Controls with X µL of distilled or deionised water. The volume X is indicated on the vial of the corresponding Quality Control. Reconstitute each Quality Control (HIGH and LOW) with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Dilute the reconstituted Quality Control at 1:3 in ELISA buffer prior to use (i.e. 100 µL Quality Control + 200 µL ELISA buffer).

The reconstituted Quality Controls must be used immediately or stored frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted (1:3) Quality Control.

▷ Wash buffer concentrated 10x

Dilute Wash Solution (10x) ten-fold in distilled water to prepare a 1x working solution, e.g. 100 ml of Wash Solution (10x) + 900 ml of distilled water for use of all 96-wells.
The diluted Wash Solution is stable for 1 month when stored at 2-8°C. Opened Wash Solution (10x) is stable 3 months when stored at 2-8°C.

▷ HRP substrate solution (TMB)

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

Opened HRP substrate solution is stable 3 months when stored at 2-8°C.

▷ 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.

Opened Stop solution is stable 3 months when stored at 2-8°C.

Assay procedure

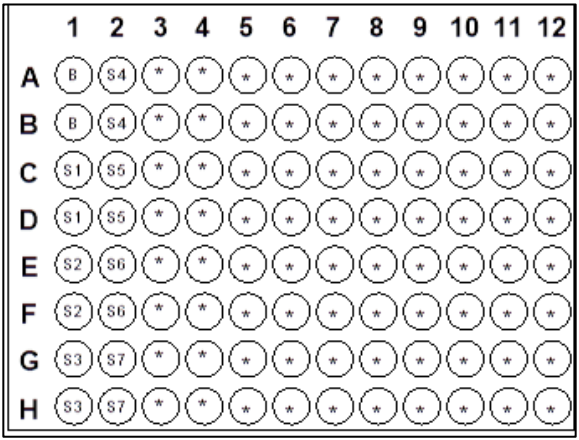
It is recommended to perform the assays in duplicate following the instructions hereafter.

Plate preparation

Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet with desiccant.
Stability at +4°C: 3 months.

Plate set-up

A plate set-up is suggested hereafter. The contents of each well may be recorded on the template sheet provided with the kit.



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

▷ Pipetting 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.

Use different tips to pipet the buffers, standards, samples, antibody and other reagents.

Before pipetting, equilibrate the pipette tips in each reagent.

Do not touch the liquid already in the well when expelling with the pipette tip.

> *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 Controls 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.

▷ Incubating the plate

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

▷ Washing the plate

Rinse each well 3 times with Wash Buffer (350 µL/well).

Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

> ***Human GFAP Biotin-labelled antibody***

Dispense 100 µL to each well.

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Washing the plate

Rinse each well 3 times with Wash Buffer (350 µL/well).

Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Pipetting the reagents

> ***Streptavidin HRP Tracer***

Dispense 100 µL to each well

Incubating the plate

Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm on an orbital microplate shaker.

Developing and reading the plate

- Empty the plate by turning it over. Rinse each well 3 times with 350 µL of Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.

- Add 100µL of HRP Substrate Solution to each well.

- Incubate the plate in the dark at room temperature without shaking for 10 to 15 minutes at room temperature.

- Add 100µL of Stop Solution to each well.

- Wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.

- Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. Read the absorbance 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.***

Assay procedure summary

Enzyme Immunoassay Protocol (volumes are in µL)			
	Blank	Standard	Sample or QC
ELISA Buffer	100	-	-
Standard	-	100	-
Sample or QC	-	-	100
Cover plate, incubate **2 hours** at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
Biotin-labelled Antibody	-	100	
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
Streptavidin HRP Tracer	100		
Cover plate, incubate **60** minutes at room temperature under orbital shaking at 300 rpm			
Wash strips 3 times with 350 µL/well Discard liquid from the wells & dry on absorbent paper			
HRP Substrate Solution	100		
Incubate the plate in the dark without agitation, during 10-15min			
Stop Solution	100		
Read the plate at 450 nm			

Data analysis

Make sure that your plate reader has subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate. If not, do it at this steps.

- 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.

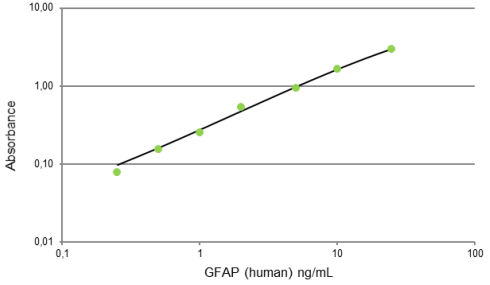
NB: Samples, Quality Controls and Standards are all diluted 3x prior to analysis, so there is no need to take this dilution factor into account.

Typical results

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.

Standard	Human GFAP ng/mL	Absorbance A.U.
S1	25.00	2.997
S2	10.00	1.648
S3	5	0.934
S4	2	0.530
S5	1	0.256
S6	0.5	0.156
S7	0.25	0.078
QC High		1.148
QC Low		0.430
Blank		0.029

Typical Human GFAP standard curve



Troubleshooting

> *Absorbance values are too low:*

- one reagent has not been dispensed,
- incorrect preparation,
- assay performed before reagents reached room temperature,
- Incorrect wavelength when reading the absorbance

> *High signal and background in all wells:*

- inefficient washing,
- overdeveloping (incubation time should be reduced),
- high ambient temperature.

> *High dispersion of duplicates:*

- poor pipetting technique
- irregular plate washing.
- Inefficient mixing Standards, Quality Controls or samples.

These are a few examples of troubleshooting that may occur. If you need further explanation, Bertin Bioreagent will be happy to assist you. Feel free to contact our Technical Support staff by phone +33 (0)139 306 036, fax +33 (0)139 306 299 or E-mail tech@bertin-bioreagent.com, and be sure to indicate the batch number of the kit (see outside the box).

Bertin Bioreagent proposes EIA Training kit #B05005 and

EIA workshop upon request. For further information, please contact our Technical Support.

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With 30 years of experience, Bertin Bioreagent develops and sells best-in-class kits and products for life science research labs. Our scientist team innovate each day to tailor biomarker assays, pre-analytical products, kits, antibodies and biochemicals that are ready to use, fully validated with a strict quality control.
We strive to address a broad range of research interest: inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, pain, prion diseases.

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