Glial fibrillary acidic protein (GFAP)

Glial fibrillary acidic protein (GFAP) is a main structural protein of astrocytes (astroglia) of the central nervous system (brain and spinal cord). It is also found in non-myelinating Schwann cells of the peripheral nervous system. It sustains the cell shape and participates in the regulation of processes related to cell proliferation, synaptic plasticity, and the function of the blood brain barrier.

Biochemistry of GFAP

GFAP belongs to a group of intermediate filament III proteins. To date, ten isoforms of GFAP have been described. However, it is only the predominant isoform GFAP-α (Isoform 1) that has been shown to have clinical significance (1).

GFAP is a fibrillar protein of approximately 50 kDa. The formation of filaments begins with the dimerization of GFAP followed by its lateral and head-to-tail polymerization. The protein is highly conserved across different species, and it is very similar to some other proteins that also participate in the formation of intermediate filaments, i.e. vimentin, desmin, peripherin and alpha-internexin.

GFAP as a marker for diagnostics

GFAP is a marker of glial cell injury. In circumstances where the glial cells are damaged, GFAP is released from cells and then appears in the blood. GFAP can be detected in blood samples shortly after the damage (2,3).

GFAP could be used as a marker for traumatic brain injury (TBI). It was shown that in the case of mild and moderate TBI, GFAP levels markedly increase eight hours after the trauma (3). The concentration of GFAP has also been suggested to predict the outcome of the injury (4). One of tests that measures GFAP (and UCH-L1) has been approved by the Food and Drug Administration for evaluating mild TBI (5).

GFAP might be also a useful biomarker for the differentiation between hemorrhagic and ischemic stroke. Both can have severe consequences, but since these two forms of strokes have opposite mechanisms, they require different strategies of treatment. Therefore, it is important to find tools that help to differentiate between these two forms of strokes as early as possible. Studies have shown that GFAP increases in the case of hemorrhagic stroke within two hours after the stroke onset, with peaking between 6 and 12 hours after the stroke onset. Instead, in the case of ischemic stroke, the GFAP levels in blood increase 24 hours after the onset of stroke (2,6).

Reagents for detecting GFAP

We provide several monoclonal antibodies (MAbs) specific to GFAP. In addition, we offer recombinant GFAP that can be used as a standard or calibrator in immunoassays.

CLINICAL UTILITY

- **Traumatic brain injury (TBI)**

MONOCLONAL ANTIBODIES SPECIFIC TO GFAP

Hytest offers several well-characterized monoclonal antibodies (MAbs) that are specific to GFAP and which may be used for the quantification of GFAP in serum, plasma or cerebrospinal fluid.

Sandwich immunoassays for GFAP detection

For the detection of GFAP in citrate or heparin plasma samples or in serum samples using a sandwich immunoassay, we recommend four different MAb combinations (see Table 1). These pairs showed no cross-reactivity to vimentin, desmin and peripherin. Calibration curves using the GFAP83cc-GFAP81cc (A) and GFAP83cc-GFAP98cc (B) prototype assays are shown in Figure 1.

Table 1.

Recommended capture-detection pairs. Data is based on the results that were obtained using a sandwich chemiluminescence immunoassay (CLIA). LoD= limit of detection.

Capture MAb	Detection MAb	LoD (pg/ml)
GFAP83cc	GFAP81cc	4.8
GFAP94cc	GFAP98cc	15.3
GFAP15cc	GFAP81cc	13.3
GFAP83cc	GFAP98cc	6.0

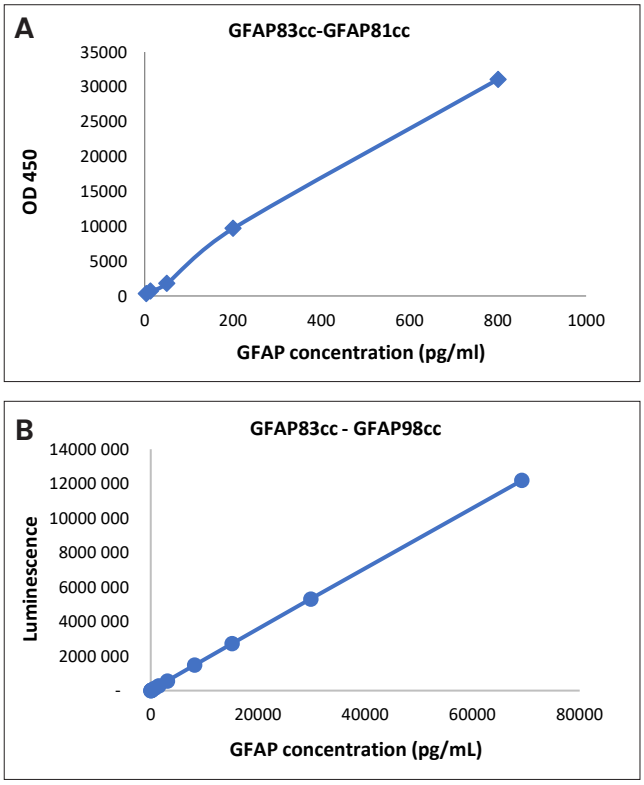

Figure 1.
Calibration curve for
A) GFAP83cc-GFAP81cc (capture-detection) pair using native GFAP as the antigen
B) GFAP83cc-GFAP98cc (capture-detection) pair using recombinant GFAP as the antigen.

Correlation with the Simoa® GFAP (Quanterix) assay

GFAP concentration in human serum samples was measured by the commercially available assay, Simoa® GFAP (Quanterix) and also with Hytest's prototype assay using pair GFAP83cc-GFAP98cc. GFAP levels were measured in 34 serum samples, with concentrations ranging from 46 - 33 074 pg/ml (as shown in Fig. 2A). The correlation curve was also constructed for the lower concentration ranging from 46 -1000 pg/mg in 18 serum samples (Fig. 2B). As shown in Figure 2, we observed a strong correlation between Hytest's prototype assay and the reference assay.

Detection of GFAP in clinical samples

Figure 3 illustrates the detection of GFAP in plasma samples that were obtained from patients with either hemorrhagic (N=5) or ischemic (N=5) stroke using the GFAP83cc–GFAP81cc assay. All of the samples were taken within the first 12 hours following the stroke onset. The prototype assay only detected GFAP in the plasma samples from patients who suffered hemorrhagic stroke. This is in line with the results from other studies and suggests that GFAP can be used for discriminating these two types of strokes. In ischemic stroke, the level of GFAP only increases in 24 hours.

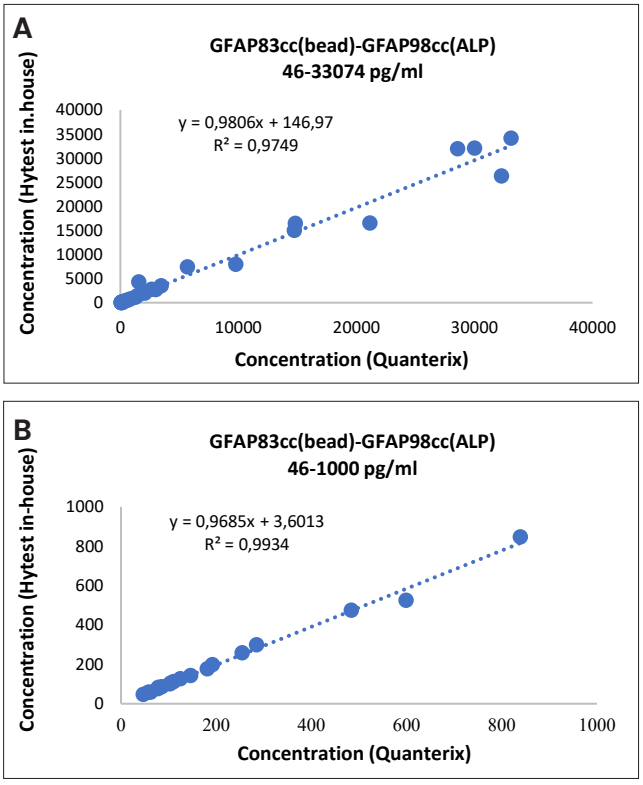

Figure 2.
Correlation between measurement results of
A) GFAP in 34 serum samples (concentrations ranging from 46-33074 pg/ml)
B) GFAP in 18 serum samples (concentrations ranging from 46-1000 pg/ml) using the GFAP83cc-GFAP98cc prototype assay, with the Simoa® GFAP (Quanterix)

MAbs suitable for immunocytochemistry

The MAbs GFAP15cc, GFAP81cc and GFAP83cc are applicable in immunocytochemistry. An example of staining GFAP in glial cells by using GFAP81cc is shown in Figure 4.

RECOMBINANT GFAP

Hytest recombinant GFAP is suitable for use as a standard or calibrator in immunoassays. The protein consists of amino acids 60-383 of human GFAP, and it is expressed in *E. coli*. Purity of the protein is over 90%. SDS-PAGE of recombinant GFAP reveals that it migrates as a single band with apparent molecular weight of 34 kDa (see Figure 5).

It should be noted that GFAP is a fibrillar protein and prone to polymerization. That is why the recombinant GFAP tends to form dimers and thus the purified protein preparation likely always contains some amount of dimeric GFAP. Long-term incubation at positive temperatures increases the percentage of the dimeric form, and can, eventually, lead to further aggregation.

Recombinant GFAP tolerates freeze-thaw cycles

We tested the immunochemical stability of recombinant GFAP after repeated freeze-thaw cycles. The immunoreactivity, as measured using GFAP83cc–GFAP81cc prototype assay, did not change significantly after the protein has been subjected to ten freeze-thaw cycles (see Figure 6).

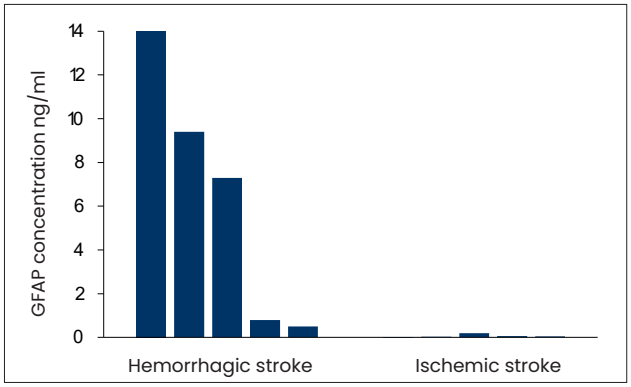

Figure 3.
GFAP was measured in plasma samples from hemorrhagic and ischemic stroke patients by using the GFAP83cc–GFAP81cc prototype assay.

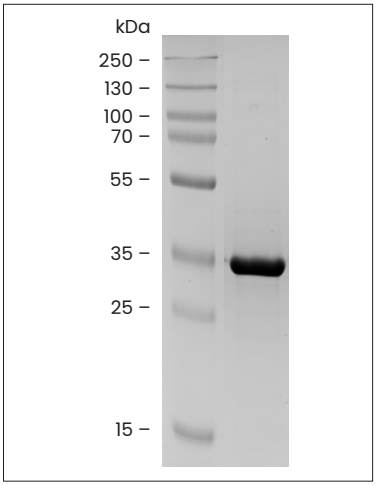

Figure 5.
SDS-PAGE of recombinant GFAP fragment under reducing conditions in a gradient gel (10-20%). 2 µg of purified protein was loaded on the gel.

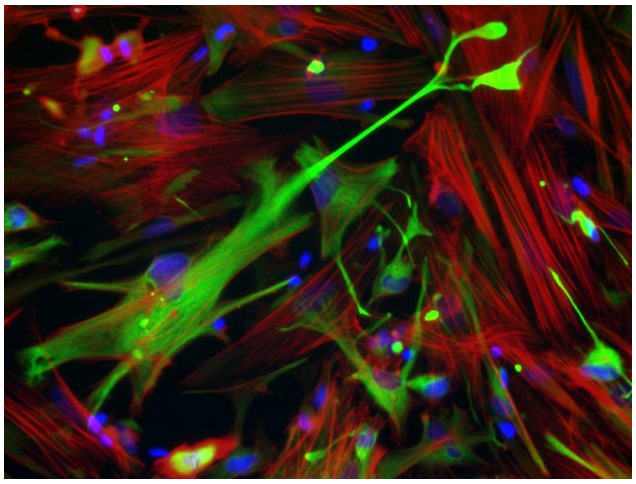

Figure 4.
Staining of GFAP in cultivated glial cells. Primary antibody: GFAP81cc. Secondary antibody: Anti-mouse polyclonal antibodies conjugated with Alexa-488 (green).

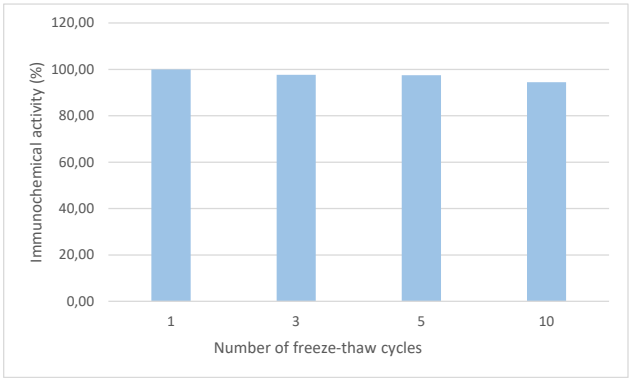

Figure 6.
Freeze-thaw stability of recombinant GFAP. Aliquot of recombinant GFAP was subjected to ten freeze-thaw cycles. Immunoreactivity after first, third, fifth and tenth cycles was measured using a sandwich immunoassay (GFAP83cc–GFAP81cc).

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Glial fibrillary acidic protein (GFAP)	4G25	GFAP15cc	IgG1	*In vitro*, EIA, WB, IHC
		GFAP81cc	IgG1	*In vitro*, EIA, WB, IHC
		GFAP83cc	IgG1	*In vitro*, EIA, WB, IHC
		GFAP94cc	IgG1	*In vitro*, EIA, WB
		GFAP98cc	IgG1	*In vitro*, EIA, WB

ANTIGENS

Product name	Cat. #	Purity	Source
Glial fibrillary acidic protein (GFAP), human, recombinant	8G47	>90%	Recombinant

HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • **HYTEST.FI**