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), and 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, as well as 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 (Isoform 1, or GFAP-α) that has been shown to have clinical significance (1).

GFAP is a fibrillar protein of approximately 50 kDa. The formation of filaments includes the lateral dimerization of GFAP and head-to-tail polymerization of the dimers that are formed. The protein is highly conserved in 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 in 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).

Marker of traumatic brain injury (TBI). Emerging evidence has shown that GFAP could be used as a TBI biomarker. It was shown that in the case of mild and moderate TBI, GFAP levels demonstrate a marked increase eight hours after the trauma (3). In addition, the concentration of GFAP has also been suggested to predict the outcome of the injury (4). Furthermore, one test that measures GFAP (and UCH-L1) has been approved by the Food and Drug Administration for evaluating mild TBI (5).

Differentiation between a hemorrhagic and an ischemic stroke. An increasing number of studies have indicated that GFAP might be a useful biomarker for the differentiation between a hemorrhagic and an ischemic stroke. Both can have severe consequences, but since these two forms of strokes have different mechanisms, they require opposite strategies of treatment. Therefore, it is important to find tools that help in terms of differentiating between the two strokes as early as possible. Studies have shown that GFAP increases in the case of a hemorrhagic stroke within two hours after stroke onset, with peaking taking place between 6 and 12 hours after stroke onset. Instead, in the case of an ischemic stroke, the GFAP levels in blood increase at a later time point (2,6).

Reagents for detecting GFAP

We provide several monoclonal antibodies (MAbs) specific to GFAP. In addition, we offer recombinant GFAP antigen 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. A calibration curve using the GFAP83cc–GFAP81cc prototype assay is shown in Figure 1.

Detection of GFAP in clinical samples

Figure 2 illustrates the detection of GFAP in plasma samples that were obtained from patients with either a hemorrhagic (N=5) or an ischemic (N=5) stroke using the GFAP83cc–GFAP81cc assay. All of the samples were taken within the first 12 hours following the injury. The prototype assay only detected GFAP in the plasma samples from patients who suffered a 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 strokes, the level of GFAP should only increase at a later time point.

MAbs suitable for immunohistochemistry

The MAbs GFAP15cc, GFAP81cc and GFAP83cc are applicable in immunohistochemistry. An example of staining GFAP in glial cells by using GFAP81cc is shown in Figure 3.

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

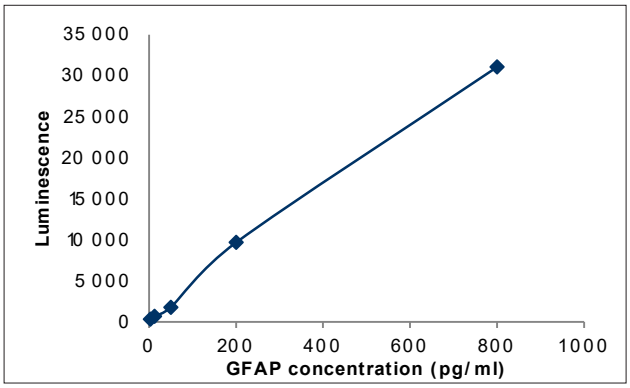

Figure 1.

Calibration curve for the GFAP83cc–GFAP81cc (capture-detection) pair using native GFAP as the antigen.

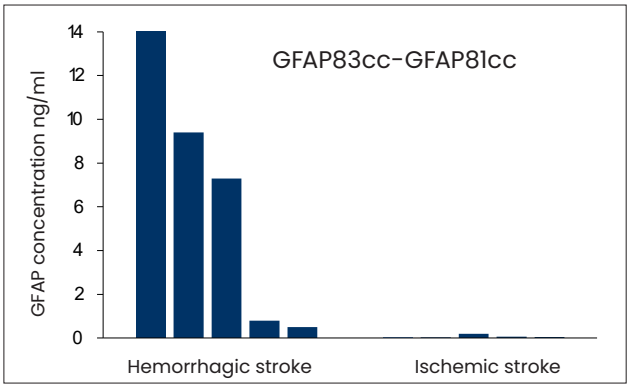

Figure 2.

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

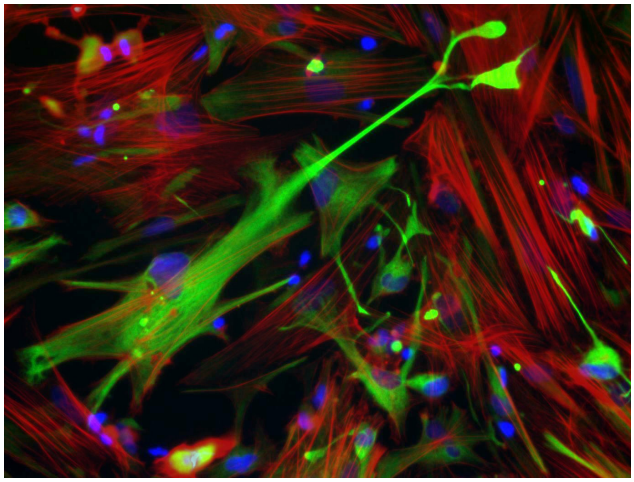

Figure 3.

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

RECOMBINANT GFAP

Hytest recombinant GFAP is suitable to be used as a standard or calibrator in immunoassays. The antigen consists of amino acids 60-383 of human GFAP and it is expressed in *E. coli*. Purity of the antigen is over 90%. SDS-PAGE of recombinant GFAP reveals that it migrates as one band with apparent molecular weight of 34 kDa (see Figure 4).

It should be noted that GFAP is a fibrillar protein and prone to polymerization. Also the recombinant GFAP has a tendency to form dimers and thus the purified protein preparation likely always contains some amount of dimeric GFAP.

To avoid precipitation of recombinant GFAP

Polymerization of GFAP may eventually lead to precipitation of the protein upon storage. Polymerization depends on conditions such as temperature, concentration and buffer used. We recommend storing the protein preparations at -70°C, reconsituting the lyophilized product to its initial concentration (always less than 1 mg/mL) and avoid using buffers with high ionic stregth such as PBS.

Recombinant GFAP tolerates freeze-thaw cycles

We tested the immunochemical stability of recombinant GFAP after repeated freeze-thaw cycles. Lyophilized protein was reconstituted in distilled water or 1% BSA. The immunoreactivity, as measured using GFAP83cc–GFAP81cc prototype assay, did not change significantly when the protein was subjected to ten freeze-thaw cycles (see Figure 5).

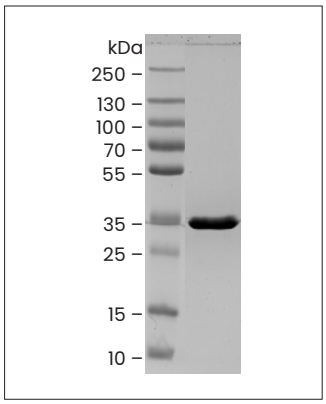

Figure 4.
SDS-PAGE of recombinant GFAP fragment under reducing conditions in a gradient gel (4-20%). *2.5 µg of purified protein was loaded on the gel.*

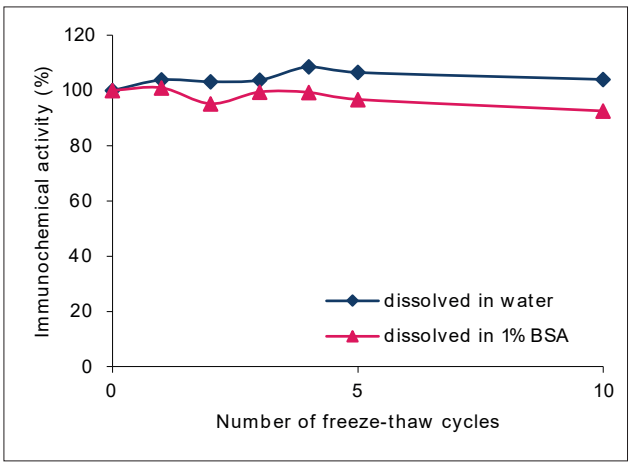

Figure 5.
Freeze-thaw stability of recombinant GFAP. *Lyophilized recombinant GFAP reconstituted to distilled water or to 1% BSA was subjected to ten freeze-thaw cycles. Immunoreactivity after each cycle was measured using a sandwich immunoassay (GFAP83cc–GFAP81cc).*

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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	8G45	>90%	Recombinant

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