

ASSAY NOTES

Product Information

Courtesy of HyTest, Ltd

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.

1. Middeldorp, J. & Hol, E. M. GFAP in health and disease. *Progress in Neurobiology* 93, 421–443 (2011).
2. Foerch, C. et al. Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke. *Clinical Chemistry* 58, 237–245 (2012).
3. Papa, L. et al. Time course and diagnostic accuracy of glial and neuronal blood biomarkers GFAP and UCH-L1 in a large cohort of trauma patients with and without mild traumatic brain injury. *JAMA Neurology* 73, 551 (2016).
4. Vos, P. E. et al. GFAP and S100B are biomarkers of traumatic brain injury: An observational cohort study. *Neurology* 75, 1786–1793 (2010).
5. U.S. Food & Drug Administration. FDA authorizes marketing of first blood test to aid in the evaluation of concussion in adults. FDA (2018). At <<http://www.fda.gov/news-events/press-announcements/fda-authorizes-marketing-first-blood-test-aid-evaluation-concussion-adults>>
6. Herrmann, M. et al. Release of glial tissue-specific proteins after acute stroke: A comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. *Stroke* 31, 2670–2677 (2000).

CLINICAL UTILITY

- Traumatic brain injury (TBI)
- Differentiate between hemorrhagic and ischemic stroke

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).

Monoclonal antibodies specific to GFAP

Advanced ImmunoChemical 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 three 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.

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	5.4
GFAP94cc	GFAP98cc	5.96
GFAP15cc	GFAP81cc	not tested

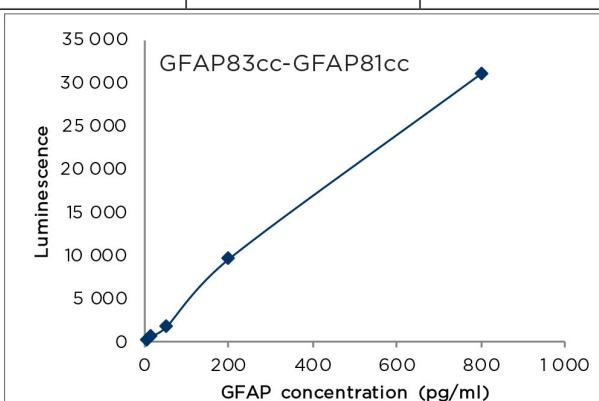


Figure 1. Calibration curve for the GFAP83cc-GFAP81cc (capture-detection) pair using native GFAP as the antigen.

Ordering Information:

MONOCLONAL ANTIBODIES

Product	Cat #	MAbs	Subclass	Remarks
Glial fibrillary acidic protein (GFAP)	2-GFAP	GFAP15cc	IgG1	<i>In vitro</i> , EIA, WB, IHC
		GFAP81cc	IgG1	<i>In vitro</i> , EIA, WB, IHC
		GFAP83cc	IgG1	<i>In vitro</i> , EIA, WB, IHC
		GFAP94cc	IgG1	<i>In vitro</i> , EIA, WB
		GFAP98cc	IgG1	<i>In vitro</i> , EIA, WB
		GF5	IgG2b	EIA, WB, IF, IHC

ANTIGEN

Product	Cat #	Purity	Source
Glial fibrillary acidic protein (GFAP)	8-GFAP-h	>90%	Human brain

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.

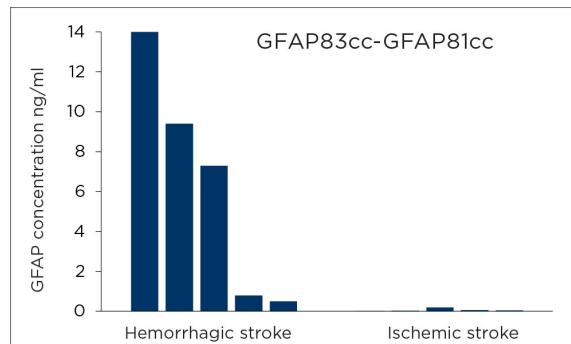


Figure 2. GFAP was measured in plasma samples from hemorrhagic and ischemic stroke patients by using the GFAP83cc-GFAP81cc prototype assay.

MAbs suitable for immunohistochemistry

The MAbs GFAP15cc, GFAP81cc, GFAP83cc and GF5 are applicable in immunohistochemistry. An example of staining GFAP in glial cells by using GFAP81cc is shown in Figure 3.

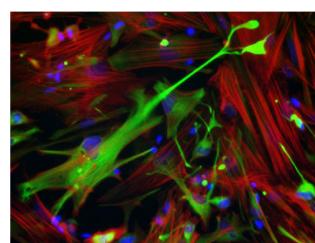


Figure 3. Staining of GFAP in cultivated glial cells. Primary antibody: GFAP81cc. Secondary antibody: Anti-mouse polyclonal antibodies conjugated with Alexa-488 (green).

