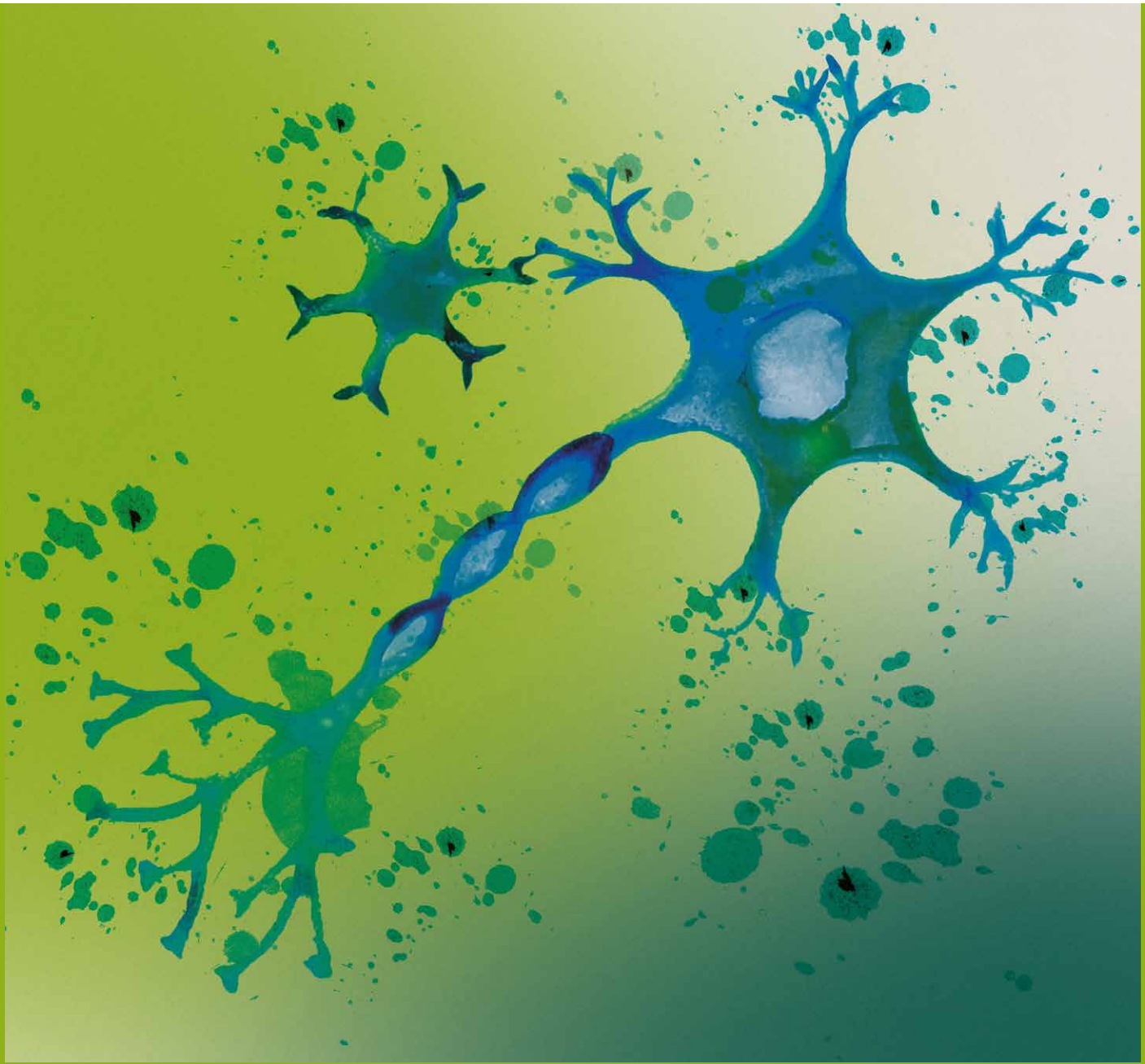
Hytest

Neuroscience

Antibodies and antigens



Introduction

Neurological conditions are currently the leading cause of ill health and disability globally, affecting more than 3 billion people worldwide. (1) Dementia is among the leading contributors to neurological health loss and is an umbrella term for several diseases affecting memory, other cognitive abilities and behaviour. Alzheimer's disease (AD) is the most common form of dementia and may contribute to 60–70% of cases. In 2021, approximately 57 million people worldwide were living with dementia. (2) The WHO Global action plan on the public health response to dementia, originally endorsed for 2017–2025, has been extended to 2031. (3).

Neurodegenerative diseases and traumatic brain injuries

Neurodegeneration is a highly complex process that is characterized by a progressive and irreversible loss of neurons from the specific brain and spinal cord regions (4). It is multifactorial and often associated with various molecular mechanisms, consequently leading to cognitive disability and dysfunctions. In neurodegenerative diseases, AD and Parkinson's disease (PD) are the most common ones. These predominantly affect the elderly population. The likelihood of developing a neurodegenerative disease rises dramatically with age.

Traumatic brain injury (TBI) and mild traumatic brain injury (mTBI) refers to injuries caused by an external force to the head. Previously, it was considered to cause a static neurological insult. Based on later studies, it has shown the potential to influence brain volume loss, which may set the stage for acute and also chronic neuroinflammatory effects, neuron damage, neurodegeneration, and dementia (5, 6). The synergistic effects of age and injury will accelerate the cognitive decline, executive dysfunction, resulting in the condition of dementia (6).

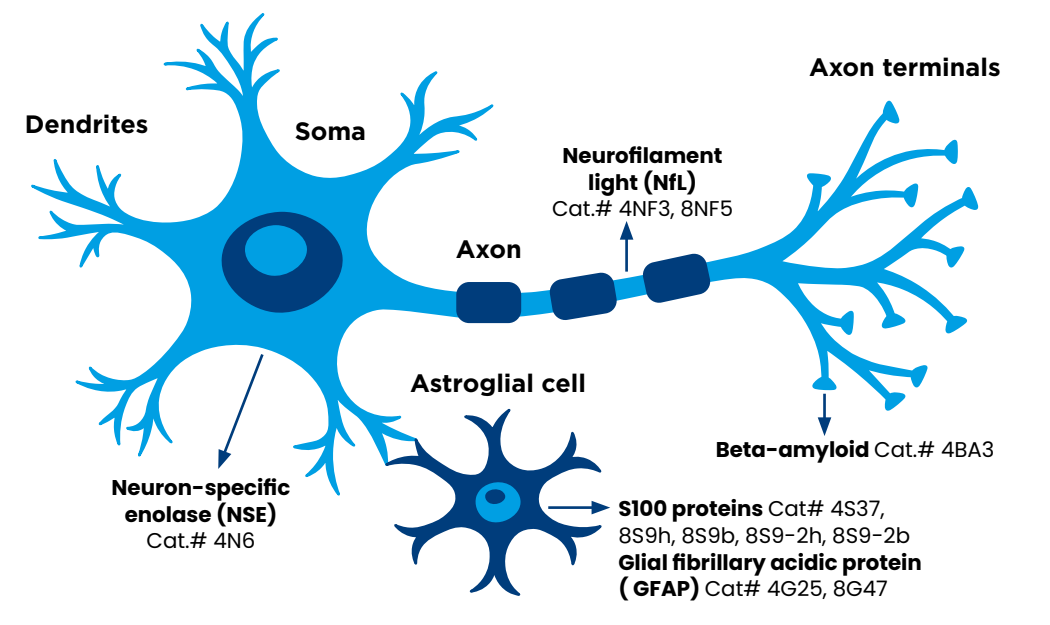


Figure 1. Neuroscience biomarkers and their locations.

The relationship between neurodegenerative diseases and TBI shows critical health implications. The risk of chronic dementia and other neurodegen-erative diseases is significantly elevated after TBI (6). Among the various neurological disorders, the biomarkers associated with the development of neurodegenerative diseases have often been relevant to TBI, such as for disease progression tracking.

Paving the way to enable early diagnosis

One of the critical challenges in terms of neurodegenerative diseases or neurological disorders is the early detection and correct identification of the causes of various neuro-logical conditions. The common diagnosis methods include cognitive tests, neuroimaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET), and detection of biomarkers from body fluids (such as cerebrospinal fluid, CSF, and blood) (6). The diagnosis of mTBIs and concussions is difficult as the imaging techniques fail to detect the case, while computed tomography (CT) may expose the patient to radiation and comes at a high cost.

Recent studies have shown the trend towards identifying novel biomarkers from samples of body fluids (4). However, diagnostic and prognostic values are still to be further discovered and fully established.

Hytest as a leading manufacturer of immunological reagents

The improvement in quality of life of older people is becoming a public health priority. As a leading reagent manufacturer, Hytest has been committed to developing high-quality immunological reagents for neurodegenerative diseases and other brain injuries since 1994. Hytest's neuroscience products include Neurofilament light (NfL), β-amyloid 42 (Aβ42), Glial fibrillary acidic protein (GFAP), and S100 proteins, which are illustrated in Figure 1.

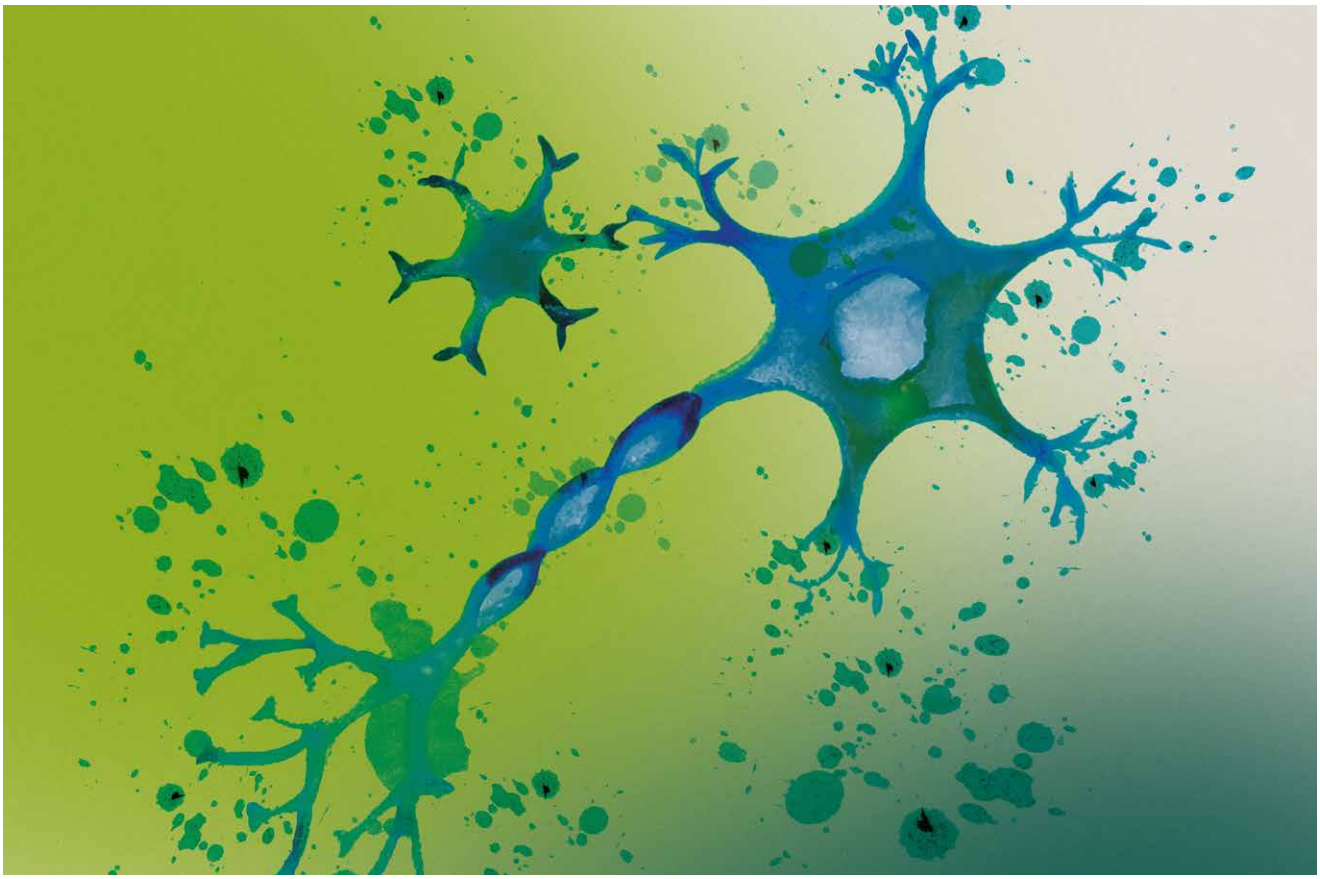
More information regarding the performance of the products and recommendations on capture-detection antibody pairs (when available) can be found on our website: www.hytest.fi

You may also contact our Sales Team directly by writing to: hytest@hytest.fi.

Abbreviations

AD	Alzheimer's disease
Aβ	β-amyloid, beta-amyloid
CLIA	Chemiluminescence immunoassay
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DALYs	Disability-adjusted life years
FDA	Food and Drug Administration
GFAP	Glial fibrillary acidic protein
Ifs	Intermediate filament
MAb	Monoclonal antibody
MRI	Magnetic resonance imaging
NfL	Neurofilament light
NSE	Neuron-specific enolase
PET	Positron emission tomography
PD	Parkinson's disease
TBI	Traumatic brain injury
mTBI	Mild traumatic brain injury
UCH-L1	Ubiquitin C-terminal hydrolase-L1
WHO	World Health Organization

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Neurofilament light (NfL)

CLINICAL UTILITY

- **Traumatic Brain Injury (TBI)**
- **Neurodegenerative diseases**

Neurofilaments are the main cytoskeletal components in neurons, which show their abundance in axons. They are bundles of 10 nm diameter filaments that are also classed as the intermediate filament (IF) proteins, which consist of a triplet subunit, including neurofilament light (NfL), neurofilament middle (NfM), and neurofilament heavy (NfH). Furthermore, there is a fourth subunit; either α-internexin in the central nervous system or peripherin in the peripheral nervous system, respectively (see Figure 2) (7).

NfL as a biomarker of neuroaxonal injury

Forming the core backbone of neurofilaments, NfL is essential to axonal structure and has emerged as a sensitive biomarker of neuroaxonal injury. Elevated NfL concentrations have been reported in both CSF and blood across a range of neurological diseases and conditions, including multiple sclerosis, amyotrophic lateral sclerosis, dementia, traumatic brain injury and stroke.

Reagents for developing reliable NfL assays

Hytest offers a range of monoclonal antibodies (MAbs) and a recombinant NfL antigen for developing sensitive and specific NfL immunoassays. MAbs recognize native NfL in CSF with high specificity and sensitivity. They show no cross-reactivity with type III IF proteins (GFAP, vimentin, desmin, peripherin) and several other neuronal proteins. Recommended MAb pairs effectively recognize both recombinant and endogenous NfL and are suitable for various applications, including direct and indirect enzyme immunoassay (EIA), sandwich immunoassays, Western blotting (WB), immunocytochemistry (ICC) and immunohistochemistry (IHC). They are also well suited for developing highly sensitive assays using technologies such as chemiluminescent immunoassay (CLIA) and single-molecule array (Simoa®).

Hytest's recombinant human NfL antigen is a highly pure and stable, full-length protein suitable for use in a wide range of immunoassays.

For more information, please refer to our NfL technotes.

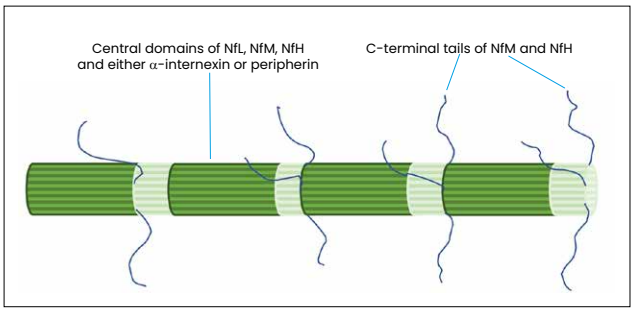
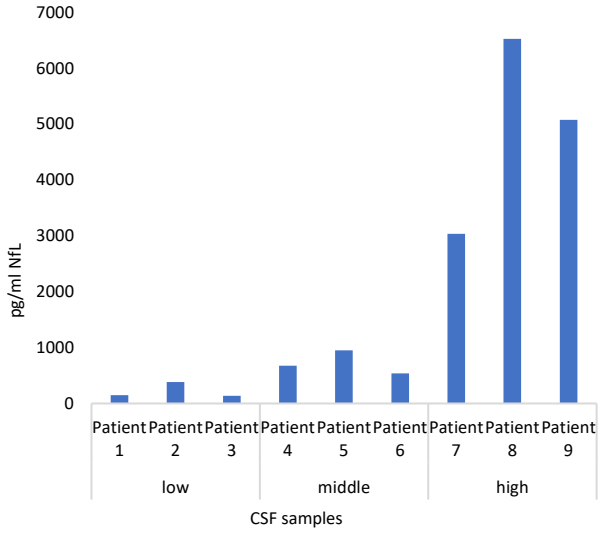


Figure 2.
Subunits of neurofilaments.

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Detection of NfL in clinical samples

A prototype assay based on Hytest's in-house pair recommendation was developed and used for the immunodetection of NfL in CSF samples from several patients with different diagnoses. NfL levels have been grouped into low, moderate, or high, as presented in Figure 3.



Patient 1	Migraine
Patient 2	Concussion
Patient 3	Pneumonia
Patient 4	Chronic cerebral ischemia
Patient 5	Unspecified stroke
Patient 6	Non-infectious encephalitis
Patient 7	Subarachnoid hemorrage
Patient 8	Dyscirculatory encephalopathy
Patient 9	Guillan-Barré syndrome

Figure 3.
NfL immunodetection in CSF samples. Prototype 3-step in-house sandwich-immunoassay NF79-NF71. The adjacent table lists the diagnosis of each patient. CSF samples were diluted 4-5-fold prior to measurement.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4NF3	Monoclonal anti-neurofilament light (NfL), human	Enzyme immunoassays

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8NF5	Neurofilament Light Chain (NfL), human, recombinant	Recombinant	≥95%

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Beta-amyloid 1-42, human

CLINICAL UTILITY

- **Alzheimer's disease (AD)**

Alzheimer's disease (AD) is a complex progressive neurodegenerative disease and it is the most common cause of dementia. The three stages of AD include preclinical Alzheimer's, mild cognitive impairment (MCI), and dementia due to Alzheimer's (10). The importance of early diagnosis has been raised in both research and clinical societies before irreversible brain damage has occurred. The accumulation of the extracellular beta-amyloid protein (which is also referred to as Aβ plaques) is one fundamental neuropathological hallmark of AD. To date, Aβ is considered as being one of the most established biomarkers for the progression of AD.

Clinical practice of β-amyloid 1-42

Appropriate use criteria for CSF β-amyloid testing Aβ42, in ratio with Aβ40, was published in 2018 (11). Besides CSF, blood-based β-amyloid has also shown promise in the diagnosis of AD in recent years (12). Among the various Aβ isoforms found in CSF, Aβ42 is considered as one of the key pathological biomarkers in terms of assessment for AD diagnosis. Aβ42 forms aggregation into fibrils and extracellular plaques in the brain, resulting in a lower level of Aβ42 in the CSF. Therefore, Aβ42 provides predictive value for AD, which can be used in both the prodromal and dementia stage of AD.

Monoclonal antibodies specific to β-amyloid 1-42

At Hytest, we provide well-characterized human β-amyloid-specific mouse MAbs for the detection of Aβ42 in human CSF. These antibodies were developed against synthetic peptides that correspond to fragments of the Aβ42 sequence. Our prototype assay immunoassays BAM7cc-BAM113cc and BAM7cc-BAM120cc correlate well with the commercially available INNOTEST βAMYLOID (142) assay, which is shown in Figure 4 on next page.

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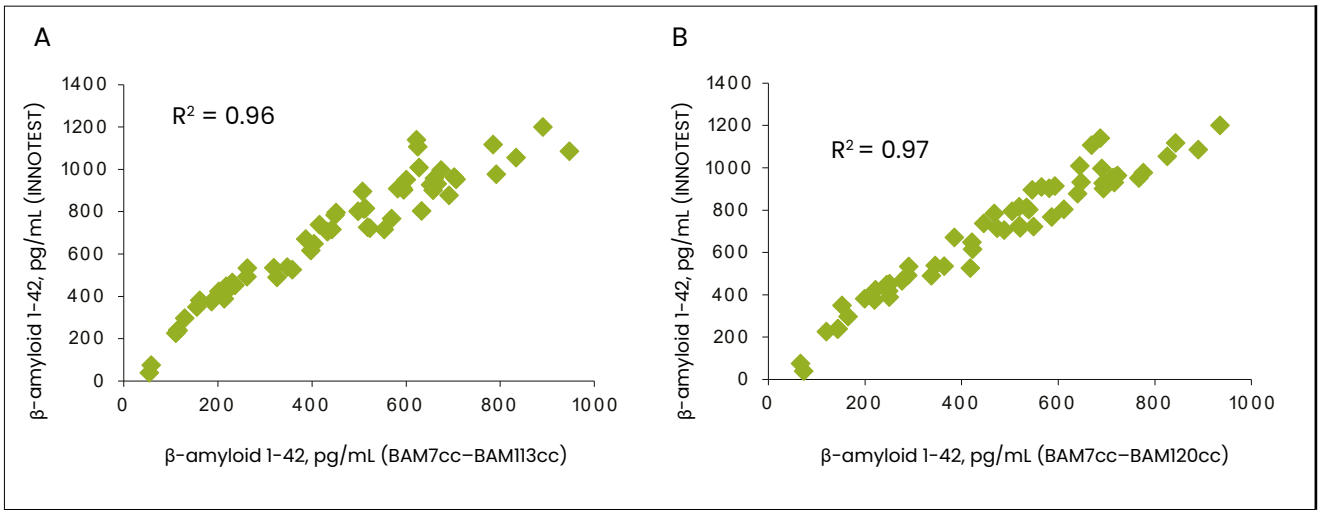


Figure 4.
Correlation studies between the immunoassays BAM7cc–BAM113cc (A) and BAM7cc–BAM120cc (B) and INNOTEST β-AMYLOID(1-42) assay. The correlation coefficients (Pearson) between the assays and the INNOTEST assay are provided in the picture.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4BA3	Monoclonal mouse anti-beta-amyloid, human	Enzyme immunoassays

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

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Glial fibrillary acidic protein (GFAP)

CLINICAL UTILITY
- **Traumatic Brain Injury (TBI)**

Glial fibrillary acidic protein (GFAP) is an astrocyte structural protein. It is a member of the type III intermediate filament that provides support and strength to cells and sustains the cell shape. GFAP proteins appear as bundled fibers and form the main intermediate filament found in specific brain cells, which are called astrocytes. Astrocytes, which are also known as astroglia, are star-shaped cells that represent approximately 30 - 40% of the cells in the central nervous system (CNS) (13). They are tightly involved in the blood-brain barrier and they have direct interactions with various cells in the nervous system.

Clinical use of GFAP

In 2018, GFAP, together with Ubiquitin C-terminal hydrolase-L1 (UCH-L1), received approval as a biomarker for concussion (mTBI) in adults by the U.S. Food and Drug Administration (FDA) (14). This represented a great milestone in the blood biomarker development for neurological disorders. Furthermore, it can be detected in serum already within the first few hours after a head injury (15).

An increasing number of studies have indicated that GFAP might be a useful biomarker for the differentiation between a hemorrhagic and an ischemic stroke. These 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 (16).

Monoclonal antibodies specific to GFAP

Hytest offers several well-characterized MAbs that are specific to GFAP and which may be used for the quantification of GFAP in serum, plasma, or CSF. We have recommendations on the capture-detection pairs using a sandwich chemiluminescence immunoassay (CLIA). For more information, please visit www.hytest.fi.

Moreover, we have tested GFAP in plasma samples that were obtained from patients with either a hemorrhagic (N=5) or an ischemic (N=5) stroke using our recommended MAb pairs (GFAP83cc–GFAP81cc). 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 had suffered a hemorrhagic stroke. This is in line with the results from other studies and it 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 stage.

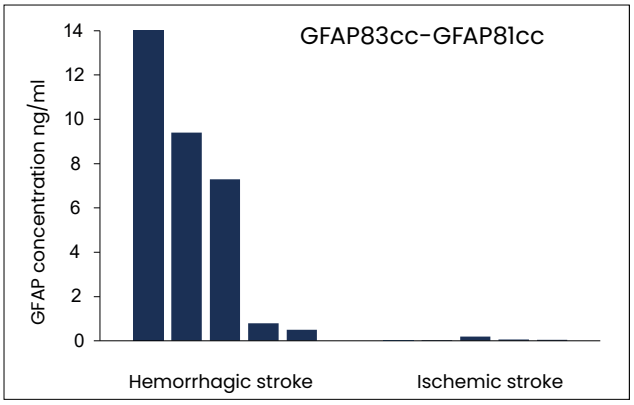


Figure 5.
GFAP was measured in plasma samples from hemorrhagic and ischemic stroke patients by using the GFAP83cc–GFAP81cc prototype assay.

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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 6).

It should be noted that GFAP is a fibrillar protein and prone to polymerization. Also, 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 percentage of 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 7).

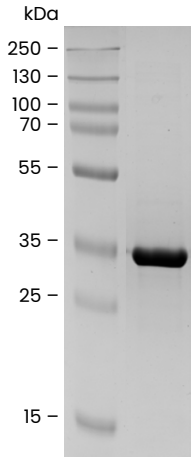


Figure 6.
SDS-PAGE of recombinant GFAP fragment under reducing conditions in a gradient gel (10-20%). 2 µg of purified protein was loaded on the gel.

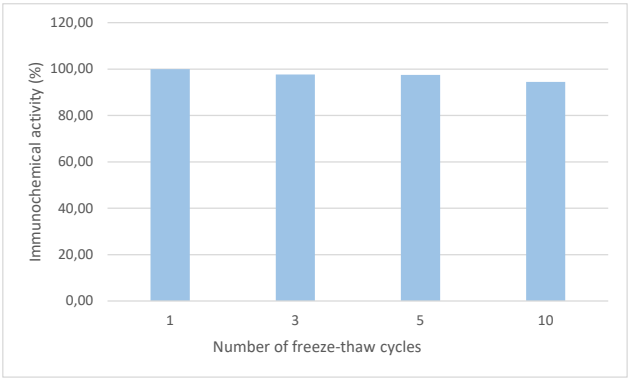


Figure 7.
Freeze-thaw stability of recombinant GFAP. Aliquot of recombinant GFAP was subjected to ten freeze-thaw cycles. Immunoreactivity after first, third, fifth and tenth cycles was measured using a sandwich immunoassay (GFAP83cc–GFAP81cc).

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4G25*	Monoclonal mouse anti-human glial fibrillary acidic protein (GFAP)	Enzyme immunoassays Western blotting Immunohistochemistry

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

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

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S100 proteins

CLINICAL UTILITY

- **Severity of brain injury and neuronal damage**
- **Traumatic and ischemic brain injury**
- **Diagnosis and prognosis of malignant melanoma**

S100 proteins constitute a family of approximately 20 calcium-binding proteins. These small proteins (10-12 kDa) have 20-50% homology of amino acid sequences but differ by origin and functions and may serve as markers of different pathological states. In brain tissue, S100 proteins are represented mainly by the S100BB homodimer and S100A1B heterodimer of approximately 21 kDa. They are synthesized in astroglial cells and can be used as sensitive and reliable markers for central nervous system damage (17).

S100 as a diagnostic marker of brain injury

Due to their abundance in the astrocytes of the brain, S100 proteins are important neuro biomarkers, such as for glial injury and blood-brain barrier damage (18, 4). They have shown elevated results over the first few days after severe TBI (16). In the Scandinavian guidelines for head injury management (19), S100B has been recommended as an option to rule out patients after mild head injuries without the need for CT. Furthermore, their involvement in relation to AD patients has also been explored (20). It plays an important role in CNS development and recovery after injury. However, their direct role in the disease mechanism is still to be fully established.

Human brain S100 proteins

Hytest's S100 proteins are purified from human brain tissue by several chromatographic methods, including gel filtration and ion exchange chromatography. After native gel electrophoresis by Ornstein-Davis, the protein is presented by two bands that correspond to A1B and BB forms (Figure 8).

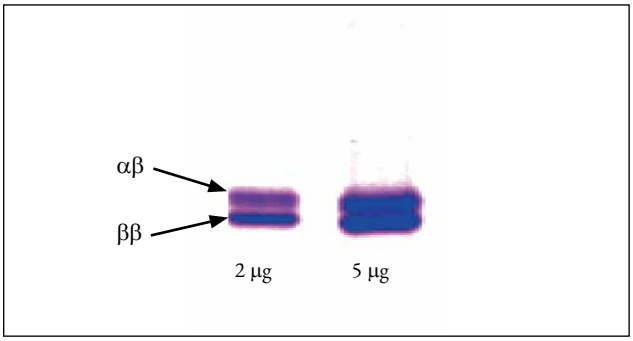


Figure 8.
Native gel electrophoresis of S100 proteins (by Ornstein – Davis).
Antigen loaded:
Lane 1: 2 µg
Lane 2: 5 µg
Gel staining: Coomassie brilliant blue R-250

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MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4S37	Monoclonal mouse anti-human S100 proteins	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8S9h	S100BB homodimer and S100A1B heterodimer, human	Human brain	>95%
8S9b	S100BB homodimer and S100A1B heterodimer, bovine	Bovine brain	>95%
8S9-2h	S100BB homodimer, human	Human brain	>95%
8S9-2b	S100BB homodimer, bovine	Bovine brain	>95%

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Additional products

Neuron-specific enolase (NSE)

Neuron-specific enolase (NSE) is a dimeric enzyme that is located primarily in the neuronal cytoplasm. NSE is the γγ homodimer isoform of the enzyme enolase. This isoform is found in neurons and cells of neuroendocrine origin as well as platelets and erythrocytes. The concentration of NSE has been shown to be elevated in serum after head injury in correlation with the degree of cell damage in CNS (21). It is a biomarker for the prognostication of neurological outcomes after cardiac arrest and has also been suggested to be a biomarker in TBI (22).

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4N6*	Monoclonal mouse anti-neuron specific enolase (NSE)	Enzyme immunoassay Western blotting Immunohistochemistry

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8NS3	Neuron-specific Enolase (NSE)	Human brain	>95%

Calmodulin

Calmodulin (CaM) is a small calcium-binding protein that plays an important role in the calcium signal transduction. It is a primary sensor, which, once bound to Ca^{2+}, undergoes a conformational change, allowing it to regulate CaM-binding proteins (23). Both CaM and its binding proteins are involved in multiple events in amyloid pathways and the tangle formation, which is a known factor for AD. CaM plays an important role in normal neuronal function and in many aspects of neurodegeneration (24).

ANTIGENS

Cat.#	Product	Source	Purity
8C10b	Calmodulin, bovine	Bovine brain	>95%
8C10h	Calmodulin, human	Human brain	>95%

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NEUROSCIENCE • ANTIBODIES AND ANTIGENS

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Hytest

Scientific excellence for IVD

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