# B-083

# Novel High Affinity Monoclonal Antibodies for Specific Detection of Neurofilament Light Protein in Serum and Cerebrospinal Fluid

Grönholm Karoliina[1], Vesilahti Ella-Maria[1], Tuomiranta Petra[1], Hämäläinen Tuula[1], Mattila Minna[1], Eklin Suvi[1]
[1]Medix Biochemica

## Introduction

Neurofilaments are very important protein components of axons, and they maintain the function of nervous system. Cerebrospinal fluid (CSF) and serum neurofilament levels elevate due to damage in nervous system making neurofilaments promising biomarkers for neural diseases and injuries, such as Alzheimer's disease, traumatic brain injury and multiple sclerosis.[1-3]

Based on their size, neurofilaments are divided in five groups: Neurofilament light (NfL), neurofilament medium (NfM), neurofilament heavy (NfH), α-internexin, and peripherin. Of these, NfL is considered the most promising biomarker since it is the most abundant form of neurofilaments.[1-3]

Medix Biochemica has developed three mouse monoclonal antibodies (mAbs) against human NfL. In this study, binding properties of these antibodies were evaluated in fluorescence-based immunoassays (FIA).

## Materials & Methods

Three anti-human NfL mAbs, designated as 12601 (#100984), 12603 (#100985) and 12604 (#100986), were assessed for their specificities and sensitivities. The antibodies were compared against commercially available reference antibodies in each FIA experiment.

Selected antibody pairs were tested in unoptimized sandwich FIA using biotin-conjugated antibodies and europium-labeled streptavidin for detection. Sensitivities, linear measuring ranges and matrix effect were studied by spiking normal serum or buffer with recombinant human NfL (#LA666). The ability of the antibodies to detect native NfL in clinical samples was determined by testing 10 CSF samples in the range of 0 to 5 083 pg/mL.

Kinetic parameters were determined with bio-layer interferometry using Octet RED96e with streptavidin biosensors, biotinylated antibodies as ligands and recombinant human NfL as the analyte.

## Results

Widest linear measuring ranges from 6 to 6 250 pg/mL in buffer samples spiked with NfL were achieved with pairs 12603 + 12601 and 12603 + 12604 with Pearson's correlation coefficients of 0.999 and 0.993, respectively (Fig. 1A). Commercial reference pair detected NfL in each sample, but Pearson's correlation coefficient was only 0.183.

Using NfL spiked normal serum as a sample, concentration range from 4 to 3 000 pg/mL could be detected with Medix Biochemica pairs 12603 + 12601, 12603 + 12604 and 12604 + 12601 with Pearson's correlation coefficients of 0.990, 0,997 and 0.972, respectively (Fig. 1B). In contrast, the commercial reference pair could not detect NfL concentrations of 12 pg/mL or 333 pg/mL leading to Pearson's correlation coefficient being only 0.860. In conclusion, matrix effect caused by serum did not affect Medix Biochemica mAbs.

With clinical CSF samples, signals achieved in FIA with pair 12604 + 12601 were well in line with the NfL levels obtained on gold-standard assay, NF-light CSF ELISA from Uman Diagnostics (Fig. 2A), with Pearson's correlation coefficient of 0.993. The signals achieved with pair 12604 + 12601 were also in line with the signals achieved with the commercial reference pair from Manufacturer A in FIA (Fig. 2B), with Pearson's correlation coefficient of 0.992.

In kinetics measurements (Table 1), none of three antibodies showed dissociation indicating very high affinity. Antibody 12603 had the fastest association of 3.8 x $10^5$ 1/Ms.

## Conclusions

This study demonstrates that the novel NfL mAbs developed by Medix Biochemica are promising tools for developing diagnostic immunoassays measuring NfL in human serum and CSF samples. Antibodies developed by Medix Biochemica performed better than the commercial reference pair in detecting native human NfL in CSF samples. Additionally, the achieved sensitivities and measuring ranges with recombinant NfL spiked normal serum indicate applicability for clinical serum samples also. Furthermore, the kinetic measurement results forecast Medix Biochemica NfL mAbs to be suitable for fast reaction kinetic assay formats, such as lateral flow, which might become a key resolution in acute neural injury diagnostics.

## Acknowledgements

We wish to thank our laboratory technicians for their excellent technical assistance in this study.

**Fig. 1. Sensitivities and linear measuring ranges of the mAb pairs were tested in sandwich FIA with recombinant human NfL spiked in A) assay buffer and B) pooled normal serum. R, Pearson's correlation coefficient. CPS, counts per second.**

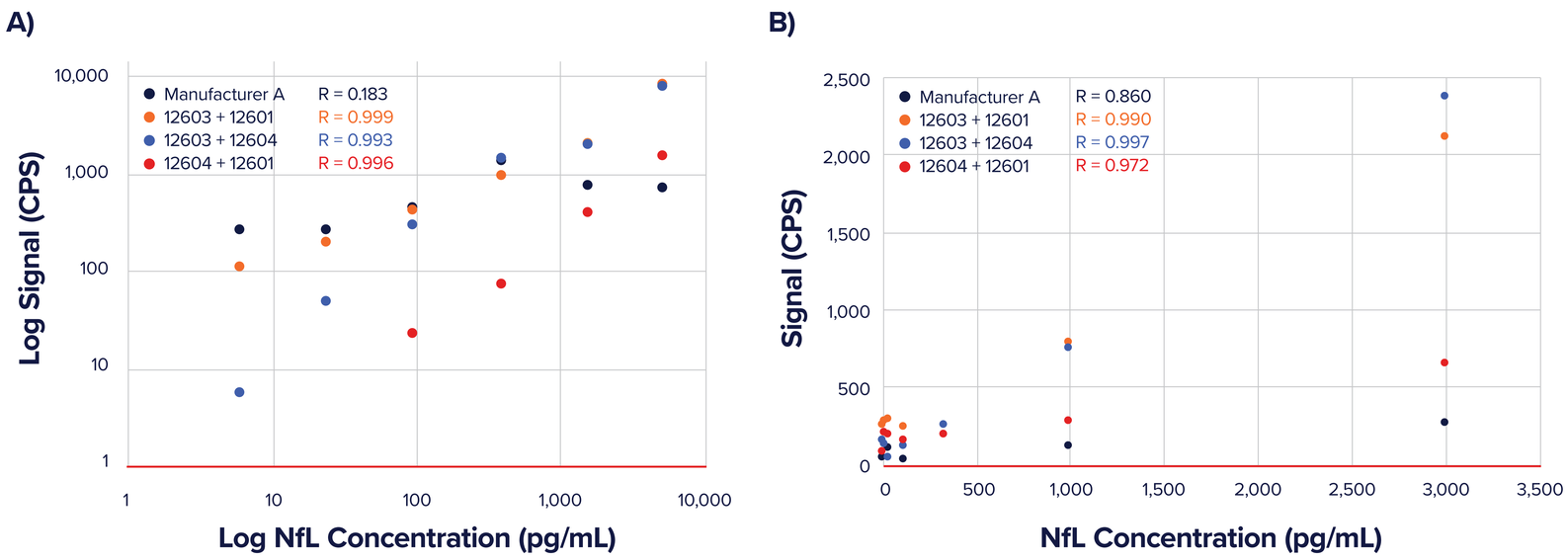


**Fig. 2. Detection of native NfL from clinical CSF samples in sandwich FIA. A) Medix Biochemica pair and commercial reference pair in FIA vs. NfL concentration determined by NF-light CSF ELISA from Uman Diagnostics. B) Medix Biochemica pair in FIA vs. commercial reference pair in FIA. R, Pearson's correlation coefficient. CPS, counts per second.**

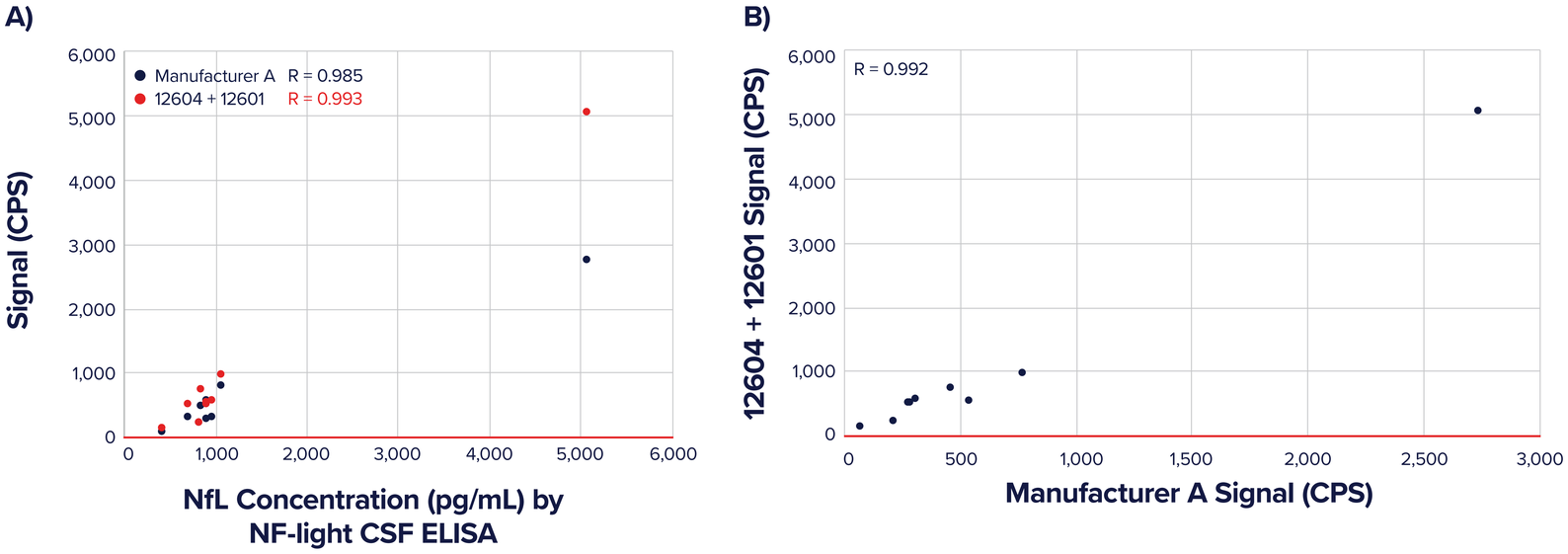


**Table. 1. Kinetic parameters of the novel NfL antibodies**

| Antibody | $k_{on}$ (1/Ms) | $k_{off}$ (1/s) | $K_D$ (M) |
|---|---|---|---|
| **12601** | 2.3 x $10^5$ | Does not dissociate under conditions used | |
| **12603** | 3.8 x $10^5$ | | |
| **12604** | 1.7 x $10^5$ | | |

**References**
[1]Gaetani et al. J Neurol Neurosurg Psychiatry, 2019. DOI: 10.1136/jnnp-2018-320106
[2]Hoyer-Kimura et al. J Neuroinflammation, 2021. DOI: 10.1186/s12974-021-02281-1.
[3]Khalil et al. Nat Rev Neurol, 2018. DOI: 10.1038/s41582-018-0058-z.

**2023 AACC**
**Annual Scientific Meeting & Clinical Lab Expo**
**July 23-27, 2023, Anaheim, California, USA**

Medix Biochemica