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Development of a novel highly sensitive mBDNF sandwich ELISA

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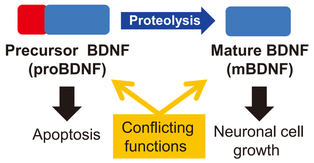
298-83901
Mature BDNF ELISA kit *Wako*, High Sensitive

Background

Brain-derived neurotrophic factor (BDNF) is a member of the NGF family of neurotrophic factors. BDNF is involved in neurogenesis and synaptogenesis and is expected to serve as a biomarker for diseases of the nervous system such as depression, autism, and schizophrenia.
BDNF has a precursor termed proBDNF, which is converted to mature BDNF (mBDNF) through the proteolytic removal of the N-terminal fragment by protease. It's important to distinguish mBDNF and proBDNF, because proBDNF has different physiological functions (apoptosis and inhibition of neurite growth) from mBDNF.

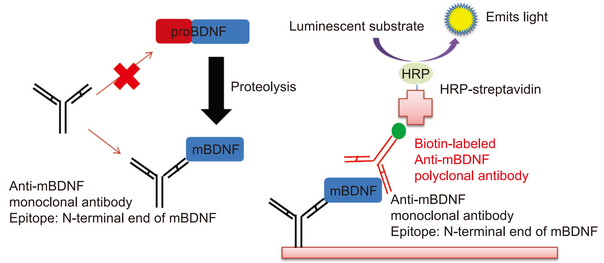


Commercially available ELISA kits can't detect mBDNF in mouse serum or plasma because of poor sensitivity and cross-reactivity with proBDNF (10-50%).

We developed a novel sandwich ELISA for mBDNF with higher sensitivity and specificity than before.

Assay principal

We constructed a sandwich ELISA which has high sensitivity (0.2 pg/mL) by using biotin-labeled, streptavidin-conjugated HRP and a luminescent substrate.
The ELISA has low cross-reactivity with proBDNF (<0.5%) because a monoclonal antibody reacts with the N-terminal end of mBDNF.



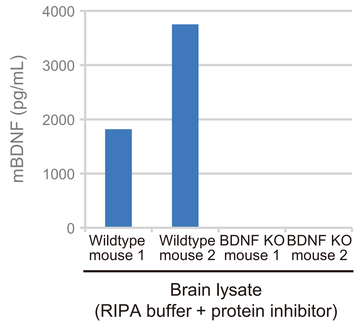
	Fujifilm Wako	Company A	Company B	Company C
Sensitivity Lowest point of Standard curve	0.2 pg/mL	62.5 pg/mL	15.6 pg/mL	15.0 pg/mL
Cross-reactivity with human proBDNF	<0.5%	Approx. 10%	Approx. 15%	Approx. 50%

Specificity

The ELISA showed low cross-reactivity with proBDNF and other NGF family proteins (NGF-β, NT-3, NT-4). In addition, the level of mBDNF detected in brain lysates of BDNF KO mice was extremely low.

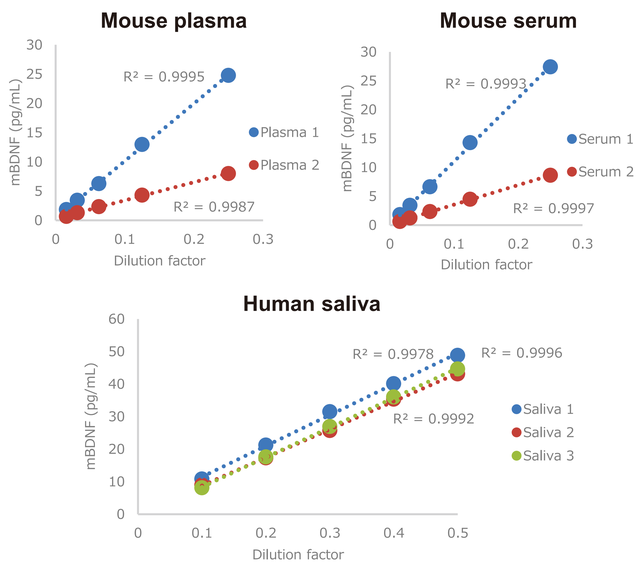
Protein	Measured value (pg/mL)	Cross-reactivity
Human proBDNF	<0.205	<0.5%
Human NGF-β	<0.205	<0.5%
Human NT-3	<0.205	<0.5%
Human NT-4	<0.205	<0.5%
Mouse proBDNF	3.31	6.62%
Mouse NGF-β	<0.205	<0.5%
Mouse NT-3	<0.205	<0.5%
Mouse NT-4	<0.205	<0.5%

50 pg/mL of the proteins were measured by the ELISA.



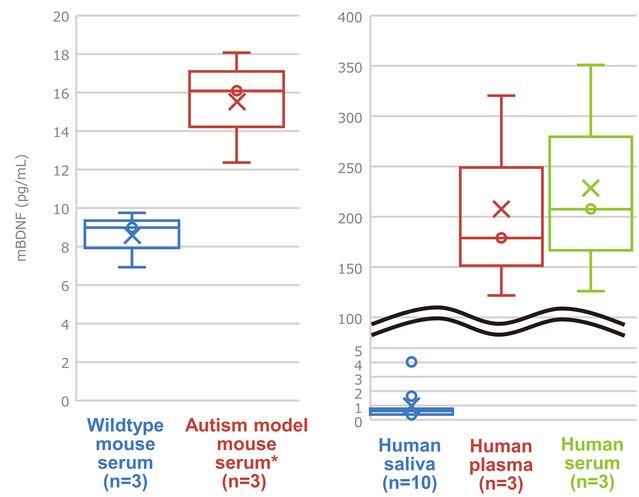
Dilution linearity

The ELISA showed good dilution linearity using mouse/rat plasma, serum, mouse brain lysate and human saliva spiked with the mBDNF. The representative data are shown.



Example of measurement

Mouse plasma mBDNF levels were measured by the ELISA. All samples showed measurable levels of mBDNF because of the high sensitivity. In addition, the mBDNF level in autism model mice tended to be higher than in wildtype mice.
Not only human plasma/serum but also saliva showed measurable levels of mBDNF. Mouse plasma and human saliva were difficult to measure using the other commercial kits because the mBDNF level was too low.



*BTBR T + Itpr3tf/J mouse

Conclusion

- We developed mBDNF specific ELISA with high sensitivity (0.2 pg/mL).
- Our novel ELISA is expected to be a useful tool in BDNF basic research and mental disease research using a model mouse and non-invasive samples.

Acknowledgements

BDNF KO mouse brain lysates were presented kindly by Dr. Masami Kojima.

Disclosure of Conflict of Interest

Matters requiring disclosures of COI with regard to the posters are as follows;
Employee of FUJIFILM Wako Pure Chemical Corporation and FUJIFILM Wako Shibayagi Corporation