"MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent

REF MF-PT1-0060

CE IVD

For *In Vitro* Diagnostic & Professional Use

Intended Use

The "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent is used to quantitatively measure Tau [pT181] levels in human fluid specimen, such as plasma. Use "MagQu" Tau protein IMR Reagent only with the XacPro-S System (MagQu Co., Ltd.).

Summary & Explanation

Tau protein is a highly soluble microtubule-associated protein (MAP) abundant in neurons mainly active in the distal portion of axon of the central nervous system which stabilize microtubules. Pathologies and dementias of the nervous system such as Alzheimer's disease and Parkinson's disease are associated with Tau protein that have become defective and no longer stabilize microtubules properly. The phosphorylated Tau protein at position threonine 181 (p-Tau181) is more specific for disease progression (from mild cognitive impairment to Alzheimer's disease).[1]

Principles of Test

The "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent is designed for quantifying Tau [pT181] by Immuno Magnetic Reduction (IMR). Anti-phospho-Tau [pT181] is conjugated on the surface of around 50 nm-in-diameter Fe_3O_4 magnetic particles. When the antibodies on the surface bind with p-Tau protein, the magnetic particles form clusters. Therefore, the ac susceptibility (Xac) of magnetic particles would be reduced in the applied ac magnetic field. By measuring the reduction of Xac, Tau [pT181] can be quantified in the sample easily and accurately.[2, 3]

Reagents

"MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent …4 x 1 mL (48 tests)

Storage Conditions & Stability

Storage reagent at 2 ~ 8 ℃ (35.6 ~ 46.4 ˚F).

Please eye check whether there is some precipitation in the tube of "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent by inverting the tube. Do not use the reagent when it has something precipitated.

Please refer to the detail expiration date on the product label.

CAUTION: Do not use reagents beyond the expiration date.

CAUTION: Do not be frozen.

Statement of Warnings

BIOHAZARD

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

Safety Data Sheet is available at www.magqu.com.

1. Do not be frozen.
2. Please keep away from events with strong magnetism.
3. For *in vitro* diagnostic use only.
4. For professional use only.
5. Do not use the reagent when it has left from 2 to 8 ℃ (35.6 to 46.4 ˚F) environment out over 24 hours.
6. Do not use the reagent when it has something precipitated.
7. Immediately after use reagent should be returned to cold storage (2 to 8 ℃).
8. Do not use reagents beyond the expiration date printed on the vial.

Reagent Preparation

1. No preparation is necessary.
2. Please use the "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagents at room temperature (15-30℃).

Specimen Collection & Preparation

BIOHAZARD

All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear facial protection, gloves, and protective clothing.

1. **Collection precautions:** Collect all blood samples by wearing protective equipment and following universal precautions for venipuncture.
2. 6 ~ 10 mL of whole blood into a blood collection tubes prepared with EDTA as an anticoagulant (Lavender Top; K2-EDTA or K3-EDTA tube).
   NOTE: Please collecting the whole blood following the manual of blood collection tube from manufacturer.
3. Invert the tube smoothly 5-10 times and make sure the whole blood specimen is mix well with EDTA.
4. Centrifuge the plasma separation tube for 15 minutes at 1,500 ~ 2,500 x g at room temperature to separate the plasma from the blood cells with swing-out(backet) rotor.
5. After centrifugation, the upper layer of plasma sample can be assayed followed by "Procedure". The plasma sample must be labeled and deep frozen (-80℃) if it is not freshly used. Avoid repeated freezing and thawing.

CAUTION: Precipitant in plasma may interfere the assay.

CAUTION: Use blood collection tubes contain K2-EDTA or K3-EDTA only. The blood collection tubes of difference brands may have a few difference substances that may influence the assay.

Procedure

Material supplied

"MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent

Materials required but not supplied

Magnetic Immunoassay Analyzer (XacPro-S)

Sample testing tubes

Transfer pipettes

1. Allow reagent and sample to reach room temperature before use.
2. Vortex them for about 5 ± 2 seconds.
3. Add 40 µL of sample into a clear sample testing tube.
4. Add 80 µL of "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent to tube.
5. Vortex them for about 5 ± 2 seconds. The rest of "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent return to 2~8℃.

6. I
nsert the sample testing tube into the measuring slot of Magnetic Immunoassay Analyzer (XacPro-S).

NOTE: Step 4 to 6 must be done within 20 minutes.

7. Process the measurement and data analysis according to the user's manual of Magnetic Immunoassay Analyzer (XacPro-S).
8. W
e suggest retesting sample if error signal (NaN) is displayed of Magnetic Immunoassay Analyzer (XacPro-S).

Performance Characteristics

Precision

The Tau [pT181] samples were measured in duplicate, once per day over 20 days. Two different Phosphorylated Tau Protein [pT181] concentrations were used for the tests. The standard deviations of repeatability and within-lab for various Tau [pT181] concentrations ware obtained:

Item tested	Mean of measured Tau [pT181] concentrations (pg/mL)	Standard deviation (%CV)	
		Repeatability	Within-Lab
pool 1	1.96	0.05786 (5.8)	0.08015 (8.0)
pool 2	22.62	0.03636 (3.6)	0.04256 (4.3)

Precision testing was determined according to CLSI/NCCLs document EP5-A3.

Interference (Specificity)

Plasma can contain interfering substances such as hemoglobin, bilirubin, or intra lipid because of common diseases, such as hemolysis, jaundice or hypertriglyceridemia. Other bio-substances that exist naturally in plasma, such as uric acid, rheumatoid factor, or albumin, are also interfering substances. Other interfering substances include drugs or chemicals in medicine that is used to treat inflammatory diseases, viral and bacterial infections, cancers and cardiovascular disease. The level of Phosphorylated Tau Protein [pT181] in each of these pools was then determined and normalized to the level without the respective substances.

Substance	Amount Added	% Recovery (Spike/control x 100)
Bilirubin	600 μg/mL	100.0
Hemoglobin	10 mg/mL	103.4
Intra lipid (Lepemis)	30 mg/mL	99.2
Albumin	60 mg/mL	99.2
Rheumatoid factor	500 IU/mL	102.1
Uric acid	200 μg/mL	95.5
Acetylsalicylic acid	500 μg/mL	91.6
Ascorbic acid	300 μg/mL	97.9
Ampicillin sodium	1 mg/mL	104.3
Rivastigmine hydrogen tartrate	100 ng/mL	102.5
Donepezil Hydrochloride	1 μg/mL	101.7
Memantine Hydrochloride	150 ng/mL	103.0
Galanthamine Hydrobromide	100 ng/mL	96.7
Quetiapine Fumarate	90 ng/mL	96.3
Human Anti-Mouse Antibody (HAMA)	100 ng/mL	105.6
Tau protein	100 pg/mL	105.6
Aβ1-40 protein	100 pg/mL	96.7
Aβ1-42 protein	100 pg/mL	106.0
α-synuclein	10 pg/mL	97.9

Interference testing was based on the principle of CLSI/NCCLs document EP7-A2.

Analytical Sensitivity

The "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent has an analytical sensitivity of 0.019569 pg/mL.

Analytical Measuring Range (AMR)

The analytical measuring range of the reagent is from 0.019569 to 100 pg/mL.

Results

By using XacPro-S, we can get two signals: one is the AC signal before the reaction (Xac_0) and the other is the AC signal after reaction (Xac). Then we can have the IMR (%) through two signals by following function:

$$IMR(\%) = \frac{Xac_0 - Xac}{Xac} \times 100$$

IMR (%), as functions of Tau [pT181] concentration $\phi_{\text{Tau [pT181]}}$ are explored and are found to follow the logistic function:

$$IMR(\%) = \frac{A - B}{1 + (\frac{\phi_{\text{Tau[pT181]}}}{\phi_o})^{\gamma}} + B$$

Where A, B, ϕ_o, and γ are fitting parameters. For Tau [pT181], A = 2.32, B = 4.43, ϕ_o = 43.83, and γ = 0.45. The IMR (%) of Tau [pT181] can be available by following equation. And you can convert to concentration by Main-analyzer.

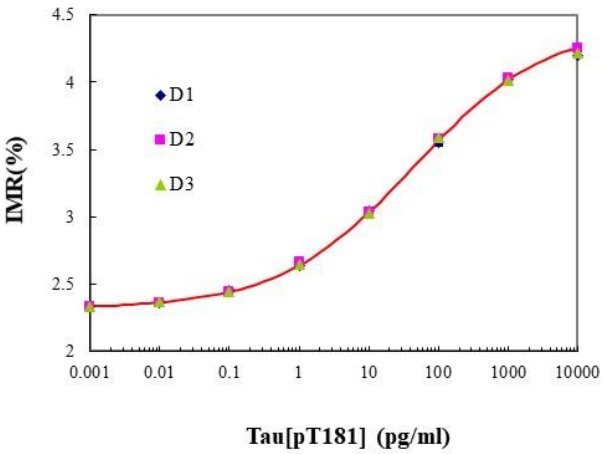

Fig.1 The IMR standard curve of Tau [pT181].

Limitations

1. The analytical range of reagent is from 0.019569 to 100 pg/mL. When the specimen with Tau [pT181] > 100 pg/mL is to be determined, carry out the following procedures to obtain the accurate concentration. Dilute the specimen, re-assay, and multiply the assayed Tau [pT181] value by the dilution factor.
2. Reagents should be used before the expiration date printed on the kit label.
3. Do not use the plasma sample when it has leaved -20 ℃ more than 2 hours or it has something precipitated.
4. Glass testing tubes are single use only.

Appendix

Clinical performance

Neuronal/axonal death in Alzheimer's disease (AD) is due to the hyperphosphorylation of Tau protein (p-Tau). There are several forms of p-Tau, depending on the phosphorylated epitopes, and they include threonine 181, serine 199, threonine 231, serine 235, serine 396, and serine 404. Cerebrospinal fluid (CSF) p-Tau level in patients with AD is significantly higher than that in healthy controls. There is a clear difference in the CSF p-Tau levels between AD and other types of dementia. The Tau protein with a phosphorylated epitope at threonine 181, which is referred as to pT181, is the subject of this work. The assay of pTau181 utilizing IMR is characterized. The "MagQu" Phosphorylated Tau Protein [pT181] IMR Reagent is used to assay pTau181 in human plasma from healthy subjects, patients with MCI due to AD, and patients with very mild AD.

Seventy-three subjects were recruited for the feasibility study of assaying pT181 in human plasma using IMR. The concentration of pT181 in plasma, φpT181 IMR, increases from the healthy controls, to patients with MCI due to AD and to patients with very mild AD. The dot plot for the measured concentrations of plasma pT181 for each subject is shown in Fig. 2. The healthy controls have a plasma pT181 of 2.46±1.09 pg/ml. The plasma pT181 level increases significantly to 4.41±1.85 pg/ml for patients with MCI due to AD ($p < 0.001$). The plasma pT181 level increases further to 6.14±1.59 pg/ml for patients with very mild AD ($p < 0.001$). [4]

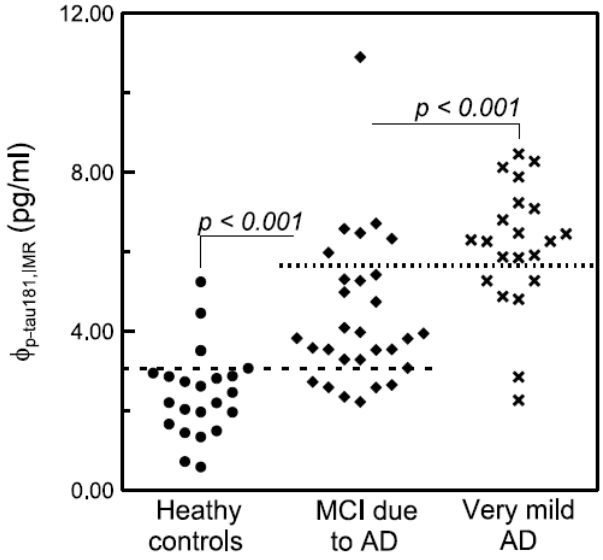

Fig.2 Plasma pT181 levels of healthy controls and patients with MCI or AD.

References

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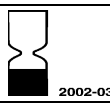
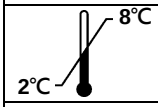
2. C.Y. Hong, C.C. Wu, Y.C. Chiu, S.Y. Yang, H.E. Horng, H.C. Yang, "Magnetic susceptibility reduction method for magnetically labeled immunoassay", *Appl. Phys. Lett.* , 88, 212512-1–212512-3, 2006.
3. C.C. Yang, S.Y. Yang, C. S. Ho, et al., "Development of antibody functionalized magnetic nanoparticles for the immunoassay of carcinoembryonic antigen: a feasibility study for clinical use" *J Nanobiotechnology*. 12: 44. 2014.
4. C.C. Yang, M.J. Chiu, T.F. Chen, H.L. Chang, B.H. Liu, S.Y. Yang, "Assay of Plasma Phosphorylated Tau Protein (Threonine 181) and Total Tau Protein in Early-Stage Alzheimer's Disease" *J Alzheimers Dis*. 61(4): 1323-1332. 2018.

Glossary/symbol definition：

SYMBOL	DESCRIPTION
	Caution, refer to accompanying documents
LOT	Batch code
REF	Catalogue number,
CONT	Content
	Use by Expressed as: CCYY-MM-DD
	Biological risk
	Consult instructions for use.
	Temperature limitation
EC REP	Authorized representative in the EU/EC.
IVD	In Vitro diagnostic medical device
	Manufacturer
	Country and date of manufacture
	Do not use if package damaged
CE	CE MARK = CONFORM WITH EEC DIRECTIVES
UDI	Unique device identifier

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