

“MagQu” α-Synuclein IMR Reagent

REF MF-ASC-0060



MagQu

For *In Vitro* Diagnostic & Professional Use

Intended Use

“MagQu” α-Synuclein IMR reagent is an *in vitro* diagnostic device intended for the quantitative determination of α-Synuclein levels to help diagnosis Parkinson's disease from human fluid specimen, such as plasma, by using the Magnetic Immunoassay Analyzer (XacPro-S).

Special instrumentation requirement

Magnetic Immunoassay Analyzer (XacPro-S)

Summary & Explanation

α-Synuclein (SNCA) is a presynaptic neuronal protein and is abundant in the human brain. α-Synuclein aggregates to form insoluble fibrils in pathological conditions characterized by Lewy bodies, such as Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). These disorders are known as synucleinopathies. α-Synuclein is the primary structural component of Lewy body fibrils. Occasionally, Lewy bodies contain tau protein; however, α-Synuclein and tau protein constitute two distinctive subsets of filaments in the same inclusion bodies. α-Synuclein pathology is also found in both sporadic and familial cases with Alzheimer's disease.^{1, 2, 3}

Principles of Test

The “MagQu” α-Synuclein IMR reagent is designed for quantifying α-Synuclein protein by Immuno Magnetic Reduction (IMR). Anti-α-Synuclein antibody is conjugated on the surface of around 50 nm-in-diameter Fe₃O₄ magnetic particles. When the antibodies on the surface bind with α-Synuclein 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, α-Synuclein protein can be quantified in the sample easily and accurately.^{4, 5}

Reagents

“MagQu” α-Synuclein IMR reagent4 x 1 mL (50 tests)

Storage Conditions & Stability

Storage reagent at 2 to 8 °C (35.6 to 46.4 °F), the shelf life is 3 months. Please eye check whether there is some precipitation in the tube of “MagQu” α-Synuclein 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 °C (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 °C).
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” α-Synuclein IMR reagents at room temperature (15-30°C).

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; 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 blood collection tubes for 15 minutes at 1,500 ~ 2,500 x g at room temperature to separate the plasma from the blood cells with swing-out(bucket) 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°C) 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 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” α-Synuclein 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 3 seconds.
3. Add 40 μL of sample to two clear sample testing tubes respectively.
4. Add 80 μL of “MagQu” α-Synuclein IMR reagent to each tubes respectively.
5. Vortex them for about 3 seconds. The rest of “MagQu” α-Synuclein IMR reagent return to 2~8°C.
6. Insert 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.

- Process the measurement according to the user's manual of Magnetic Immunoassay Analyzer (XacPro-S).
- Use the logistic function or table 1 for converting to the concentration of α -Synuclein protein.
- Calculate the average value for each concentration. Concentration of α -Synuclein protein is determined.
- We suggest retesting sample if Analyzer (XacPro-S) displays error signal (NaN) or the coefficient of variation (CV, %) is above 30 %.

$$\text{Coefficient of Variation (CV, \%)} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Performance Characteristics

Precision

The α -Synuclein samples were measured in duplicate, once per day over 20 days. The two measurements on two sequent days are regarded as two runs. Two different α -Synuclein concentrations were used for the tests. The standard deviations of repeatability and within-lab for various α -Synuclein concentrations were obtained:

Item tested	Mean of measured α -Synuclein concentrations (pg/mL)	Standard deviation (%CV)	
		Repeatability	Within-Lab
Sample 1	0.0154	0.00132 (8.6)	0.00213 (13.8)
Sample 2	1.0261	0.08700 (8.5)	0.11312 (11.0)

Precision testing was determined according to CLSI/NCCLS document EP5-A2.

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 α -Synuclein in each of these pools was then determined and normalized to the level without the respective substances.

Substance	Amount Added	Mean % Recovery (Spike/control x 100)
Hemoglobin	10000 μ g/mL	95.94
Conjugated bilirubin	600 μ g/mL	92.31
Intra lipid	30000 μ g/mL	90.45
Uric acid	200 μ g/mL	104.05
Rheumatoid factor	500 IU/mL	100.83
Albumin	60000 μ g/mL	102.82
Acetylsalicylic acid	500 μ g/mL	99.36
Ascorbic acid	300 μ g/mL	107.95
Ampicillin sodium	1000 μ g/mL	104.22
Levodopa	1000 μ g/mL	96.52
Quetiapine Fumarate	100 ng/mL	105.02
Galantamine hydrobromide	90 ng/ml	100.10
Rivastigmine hydrogen tartrate	100 ng/ml	96.18
Donepezil Hydrochloride	1000 ng/ml	91.67
Memantine Hydrochloride	150 ng/ml	99.18
Human Anti-Mouse Antibody(HAMA)	100 ng/ml	106.25
Human Anti-Mouse Antibody(HAMA)	1000 ng/ml	109.86

Interference testing was determined according to CLSI/NCCLS document EP7-P.

Analytical Sensitivity

The "MagQu" α -Synuclein IMR reagent has an analytical sensitivity of 0.001396 pg/mL.

Analytical Measuring Range (AMR)

The analytical measuring range of the reagent is from 0.001396 to 1,020 pg/mL.

Results

By using Magnetic Immunoassay Analyzer (XacPro-S), two signals of each assay are acquired: one is the AC signal before the reaction ($X_{ac,o}$) and the other is the AC signal after reaction ($X_{ac,\phi}$). Then we can have the IMR (%) through two signals by following function:

$$IMR(\%) = \frac{X_{ac,o} - X_{ac,\phi}}{X_{ac,o}} \times 100\%$$

IMR (%), as functions of α -Synuclein protein concentration $\phi_{\alpha\text{-Synuclein}}$ are explored and are found to follow the logistic function:

$$IMR(\%) = \frac{A - B}{1 + \left(\frac{\phi_{\alpha\text{-Synuclein}}}{\phi_o}\right)^\gamma} + B$$

Where A, B, ϕ_o , and γ are fitting parameters. For α -Synuclein, A = 2.78, B = 8.39, ϕ_o = 1553.54, and γ = 0.21. The concentration of α -Synuclein protein can be available by following equation. And you can convert to concentration by Main-analyzer.

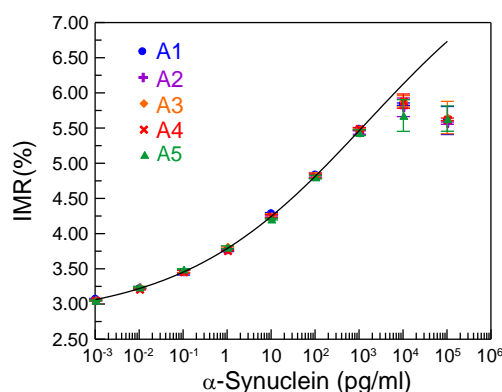


Fig.1 IMR standard curve of α -Synuclein.

Limitations

- The analytical range of reagent is from 0.001396 to 1,020 pg/mL. When the specimen with α -Synuclein protein > 1,020 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 α -Synuclein protein value by the dilution factor.
- Reagents should be used before the expiration date printed on the kit label.
- Do not use the plasma sample when it has leaved -20 $^{\circ}$ C more than 2 hours or it has something precipitated.
- Sample testing tubes are single use only.

Appendix

Clinical performance

Parkinson's disease (PD) is characterized by the presence of Lewy bodies containing aggregated α -synuclein, which is also present in human body fluids including cerebrospinal fluid (CSF) and blood plasma. Accordingly, the majority of body fluid biomarker studies have studied CSF samples of patients with PD, either to assess disease risk or to predict cognitive deterioration. However, thus far, results have been inconsistent other than to identify a consistently lower level of α -synuclein in PD patients with dementia (PDD) versus PD patients with normal cognition. The relatively invasive procedure for collecting CSF has led to investigations of serum or plasma as alternatives. Currently, CSF, serum or plasma biomarkers are mainly analyzed using ELISA, or similar immunoassay techniques. These assays are often performed manually and are therefore difficult to standardize, which has resulted in substantial variability in measurements between clinical centers and laboratories. Plasma levels of α -synuclein are also exceptionally low compared with CSF, which has hampered the use of ELISA in accurately detecting plasma α -synuclein.⁶

The plasma α -synuclein was significantly increased in patients with PD with normal control subjects.

By measuring α -synuclein in plasma by α -synuclein IMR reagent, plasma levels of α -synuclein were significantly higher in patients with PD (n=80) compared with controls (n=34) (Fig.2, median: 1.56 pg/mL, 95% CI 1.02 to 1.98 pg/mL vs 0.02 pg/mL, 95% CI 0.01 to 0.03 pg/mL; $p < 0.0001$).⁶ When cut-off value is 0.1161 pg/ml, the sensitivity, specificity and area under curve (AUC) are 0.857, 0.971 and 0.932, respectively.

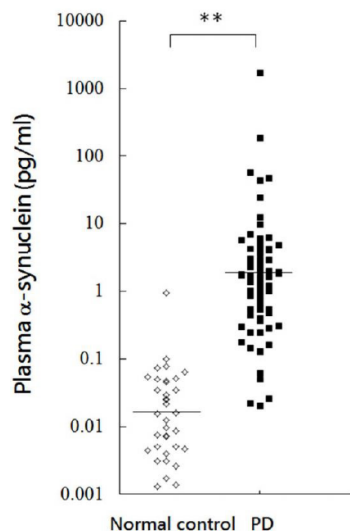




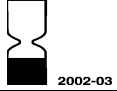


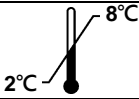







Fig.2 Plasma α -synuclein levels of normal controls and patients with PD.

References

1. A. Atik, T. Stewart, J. Zhang, "Alpha-synuclein as a biomarker for Parkinson's disease", *Brain Pathology*, Volume 26, Issue 3, pages 410–418, May 2016
2. O. Hansson, S. Hall, A. Öhrfelt, H. Zetterberg, K. Blennow, L. Minthon, K. Nägga, E. Londos, S. Varghese, N.K. Majbour, A.Al-Hayani, O.M.El-Agna, "Levels of cerebrospinal fluid α -Synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease", *Hansson et al. Alzheimer's Research & Therapy*, 2014, 6:25.
3. D.J. Irwin, V.M.Y. Lee, and J.Q. Trojanowski, "Parkinson's disease dementia convergence of α -Synuclein, tau and amyloid- β pathologies", *Nat Rev Neurosci.*, 14, 626–636, 2013.
4. 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.
5. 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.", *Journal of Nanobiotechnology*, 2014, 12:44.
6. C.H. Lin, S.Y. Yang, H.E. Horng, C.C. Yang, J.J. Chieh, H.Hsien C., B.H. Liu, M.J. Chiu, "Plasma α -synuclein predicts cognitive decline in Parkinson's disease.", *et al. J Neurol Neurosurg Psychiatry*, 2017;0:1–7.

Glossary/symbol definition :

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



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