

## "MagQu" Phospho- $\alpha$ -synuclein IMR Reagent

**REF** MF-PS1-0060



For *In Vitro* Diagnostic & Professional Use

### Intended Use

The "MagQu" Phospho- $\alpha$ -synuclein IMR Reagent is used to quantitatively measure phosphorylated  $\alpha$ -synuclein in human fluid specimen, such as plasma. Use "MagQu" Phospho- $\alpha$ -synuclein IMR Reagent only with the XacPro-S System (MagQu Co., Ltd.).

### Summary & Explanation

Parkinson's disease (PD) is characterized by the intraneuronal  $\alpha$ -synuclein inclusions called Lewy bodies. Increase of phosphorylation of  $\alpha$ -synuclein in Serine 129 (S129) has been correlated with the aggregation, toxicity, protein interaction and turnover of  $\alpha$ -synuclein. Thus, S129 can indicate the pathogenesis of PD.<sup>1,2</sup>

### Principles of Test

The "MagQu" Phospho- $\alpha$ -synuclein IMR Reagent is designed for rapid quantifying phosphorylated  $\alpha$ -synuclein (S129) by ImmunoMagnetic Reduction (IMR). We conjugate antibody on the surface of around 50 nm-in-diameter Fe<sub>3</sub>O<sub>4</sub> magnetic particles. When the antibodies on the surface bind with phosphorylated  $\alpha$ -synuclein (S129), the magnetic particles form clusters. Therefore, the ac susceptibility (Xac) of magnetic particles would be reduced in the adding ac magnetic field. By measuring the reduction of Xac, phosphorylated  $\alpha$ -synuclein (S129) can be easily, rapidly and accurately quantified.

### Reagents

"MagQu" Phospho- $\alpha$ -synuclein IMR Reagent ...4 x 1 mL (65 tests)

### Storage Conditions & Stability

Storage reagent at 2 ~ 8 °C (35.6 ~ 46.4 °F).

Please eye check whether there is some precipitation in the tube of "MagQu" Phospho- $\alpha$ -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](http://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" Phospho- $\alpha$ -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" Phospho- $\alpha$ -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 60  $\mu$ L of sample into two clear sample testing tubes respectively.
4. Add 60  $\mu$ L of "MagQu" Phospho- $\alpha$ -synuclein IMR Reagent to each tubes respectively.
5. Vortex them for about 3 seconds. The rest of "MagQu" Phospho- $\alpha$ -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.
7. Process the measurement and data analysis according to the user's manual of Magnetic Immunoassay Analyzer (XacPro-S).
8. Calculate the average value for each concentration.
9. We suggest retesting sample if error signal (NaN) is displayed of

Magnetic Immunoassay Analyzer (XacPro-S) or coefficient of variation (CV, %) above 20%.

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

## Performance Characteristics

### 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. The level of phosphorylated  $\alpha$ -synuclein (S129) 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 $\mu\text{g/mL}$	91.0
Conjugated bilirubin	600 $\mu\text{g/mL}$	91.7
Intra lipid	30000 $\mu\text{g/mL}$	95.8
Uric acid	200 $\mu\text{g/mL}$	96.7
Rheumatoid factor	500 IU/mL	103.3
Albumin	60000 $\mu\text{g/mL}$	97.2
$\alpha$ -synuclein	1 $\text{pg/mL}$	105.4

### Analytical Sensitivity

The "MagQu" Phospho- $\alpha$ -synuclein IMR reagent has an analytical sensitivity of 0.072 fg/mL.

### Analytical Measuring Range (AMR)

The analytical measuring range of the reagent is from 0.00048 to 48.26 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 phosphorylated  $\alpha$ -synuclein (S129) concentration  $\phi$  p-ASC129 are explored and are found to follow the logistic function:

$$IMR(\%) = \frac{A - B}{1 + \left(\frac{\phi_{p-ASC129}}{\phi_o}\right)^\gamma} + B$$

where A, B,  $\phi_o$ , and  $\gamma$  are fitting parameters. For phosphorylated  $\alpha$ -synuclein (S129), A = 2.636, B = 4.138,  $\phi_o$  = 0.635, and  $\gamma$  = 0.602.

The concentration of phosphorylated  $\alpha$ -synuclein can be available by Main-analyzer.

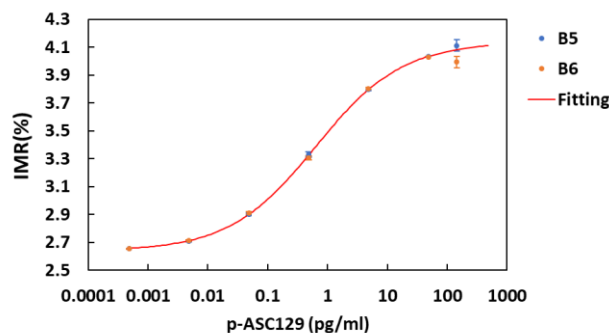


Fig.1 The IMR standard curve of phosphorylated  $\alpha$ -synuclein (S129)





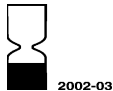


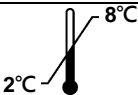


## Limitations


1. The analytical range of reagent is from 0.00048 to 48.26 pg/mL. When the specimen with phosphorylated  $\alpha$ -synuclein (S129) > 48.26 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 phosphorylated  $\alpha$ -synuclein (S129) value by the dilution factor.
2. Reagents should be used before the expiration date printed on the kit label.
3. Data is based upon human plasma sample.
4. Do not use the plasma sample when it has leaved  $-20\text{ }^\circ\text{C}$  more than 2 hours or it has something precipitated.
5. Glass testing tubes are single use only.

## References

1. Wang, Y., Shi, M., Chung, K. A., Zabetian, C. P., Leverenz, J. B., Berg, D., Srujijes, K., Trojanowski, J. Q., Lee, V. M., Siderowf, A. D., Hurtig, H., Litvan, I., Schiess, M. C., Peskind, E. R., Masuda, M., Hasegawa, M., Lin, X., Pan, C., Galasko, D., Goldstein, D. S., Jensen, P. H., Yang, H., Cain, K. C., Zhang, J. (2012). Phosphorylated  $\alpha$ -synuclein in Parkinson's disease. *Science translational medicine*, 4(121), 121ra20.
2. Oueslati A. (2016). Implication of Alpha-Synuclein Phosphorylation at S129 in Synucleinopathies: What Have We Learned in the Last Decade?. *Journal of Parkinson's disease*, 6(1), 39-51.

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
	Manufacturer
	Do not use if package damaged

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