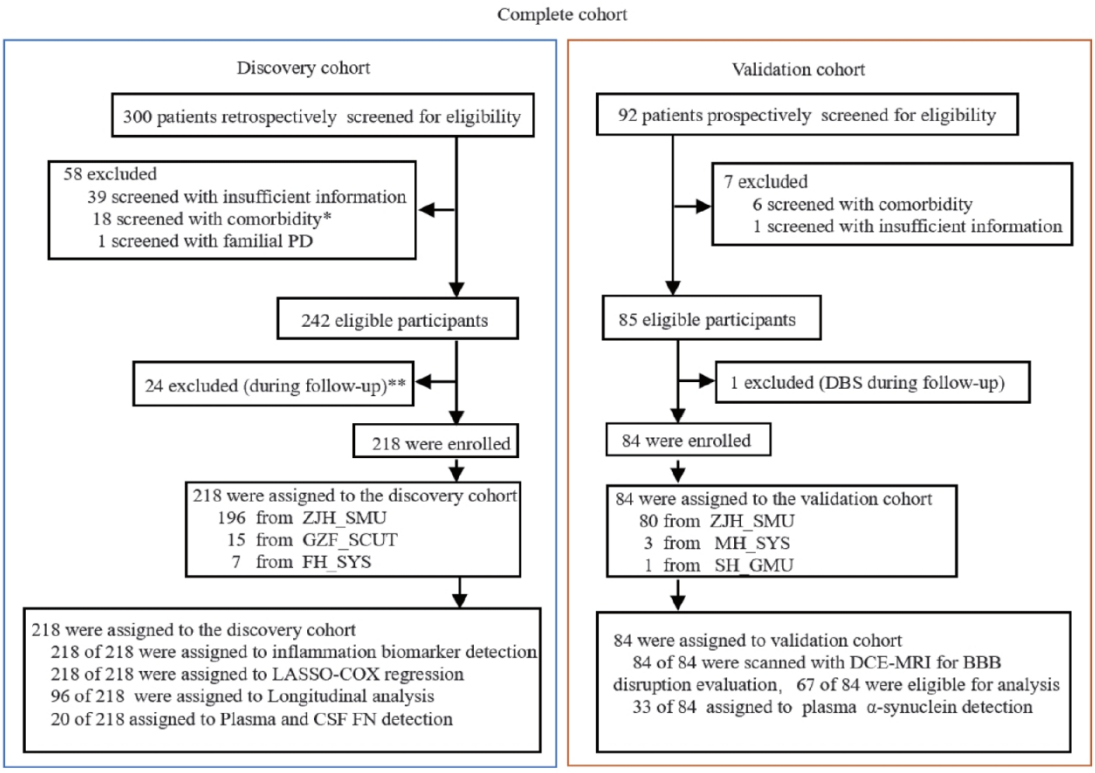
Supplementary data

Inclusion and exclusion criteria

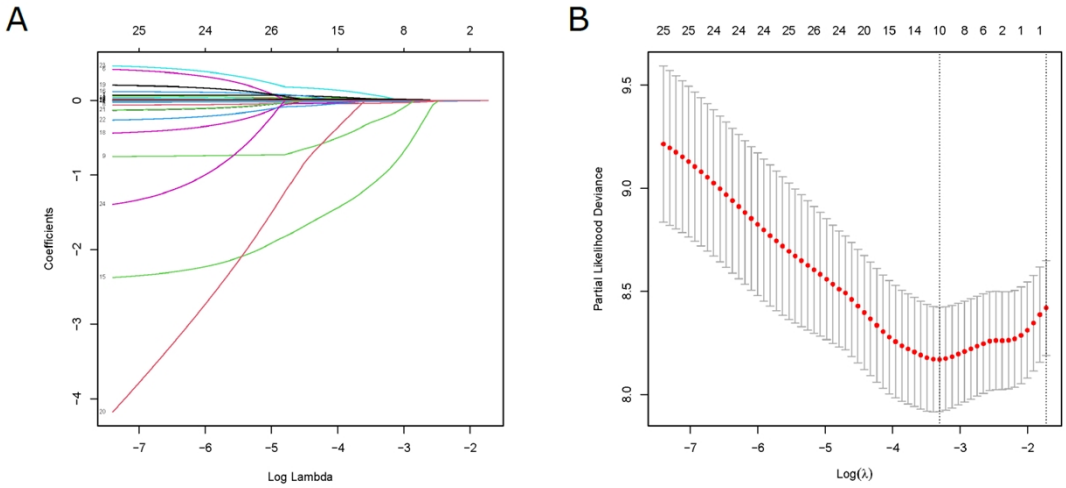
The inclusion criteria were as follows: (1) 18–85 years old; (2) PD without any disability milestones (i.e., frequent falling, wheelchair dependence, residential care, dementia, visual hallucinations and death); and (3) agreement to participate in the clinical trial. The exclusion criteria were comorbidities that were likely to affect the evaluation of neurological function or disease progression, as follows: (1) PD patients with neurological comorbidities, including Alzheimer's disease (AD), major psychiatric disorders, cerebrovascular disease, head tumor or injury, and normal pressure hydrocephalus; (2) PD patients with internal comorbidities, including systemic infection, liver or kidney disease, cardiovascular events, tumors, hematemesis, other critical diseases and severe organ function failure; (3) PD patients with a history of surgery or trauma, including deep brain stimulation (DBS) operation and bone fracture; and (4) PD patients with contraindications to magnetic resonance (MR) examination, including a). Patients with cardiac pacemakers and cardiac stents; b). Patients with claustrophobia and pregnancy; c). Patients with fixed dentures, birth control devices, etc.; and d). Patients allergic to magnetic resonance contrast agents.

Supplementary Figure 1. Flow chart of the study



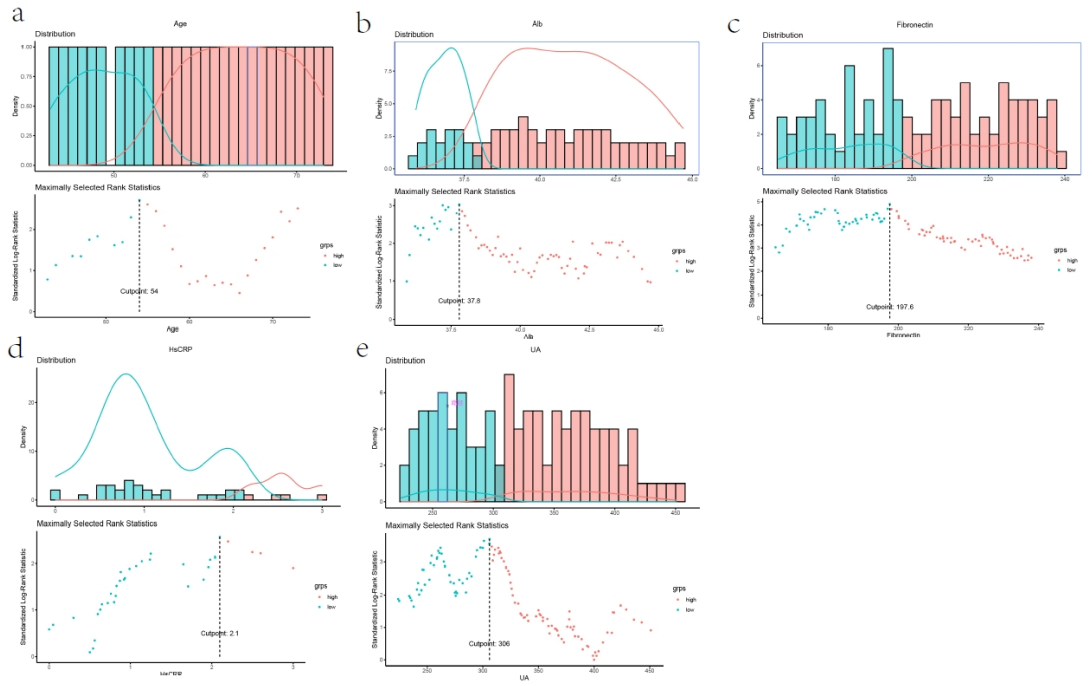
Flow diagram illustrating the design of the study. Abbreviations: CSF= Cerebrospinal Fluid; DBS= deep brain stimulation; DCE-MRI: dynamic contrast-enhanced magnetic resonance imaging; FN= fibronectin; LASSO-COX= Least Absolute Shrinkage and Selection Operator – Cox; PD= Parkinson's disease; ZJH_SMU: Zhujiang Hospital of Southern Medical University; MH_SYS: Sun Yat-Sen Memorial Hospital of Sun Yat-sen University; FH_SYS: The First Affiliated Hospital of Sun Yat-sen University; GZF_SCUT: Guangzhou First People's Hospital of South China University of Technology; SH_GMU: The Second Affiliated Hospital of Guangzhou Medical University. ** 24 excluded (lost to follow-up) in the discovery cohort. *8 with serious cardiovascular and cerebrovascular diseases; 2 with DBS; 2 with cancer; 5 with bone fracture; 7 lost to follow-up or conflicting clinical information.

Supplementary Figure 2. Possible risk factors related to the development of the first milestone of PD patients screened by Lasso-Cox regression



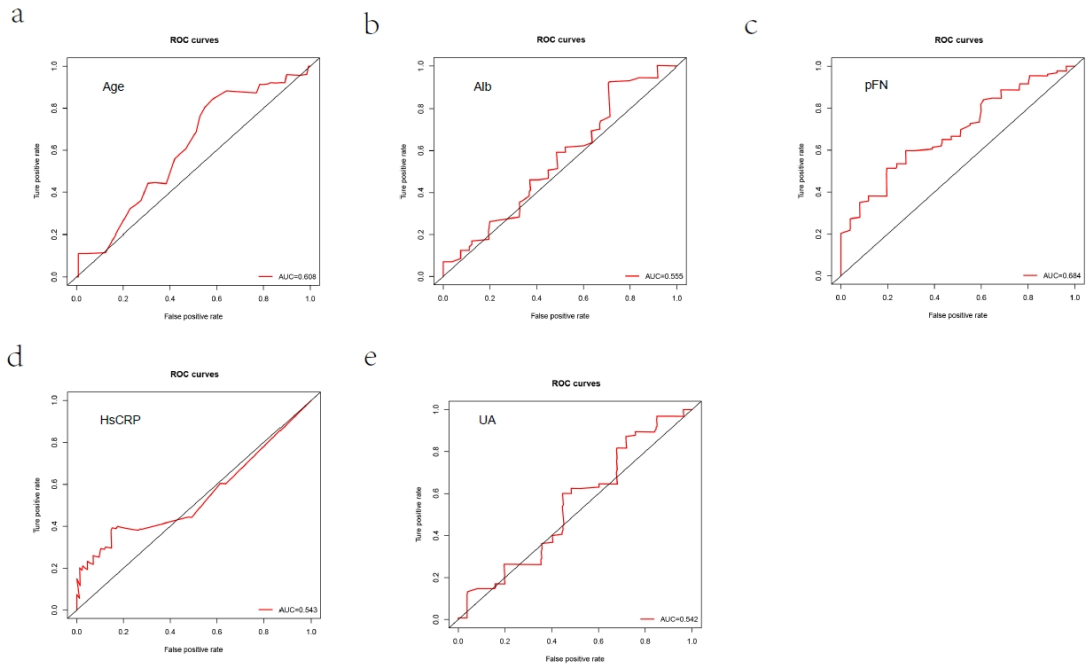
Regression coefficient diagram based on LASSO Cox regression. **(A)** LASSO coefficient profiles for several variables in univariate linear regression analysis. Coefficient profiles decrease with larger lambda values. **(B)** Cross-validation for selecting the tuning parameters for the LASSO model. The vertical lines are plotted based on the optimal data according to the minimum criteria and 1-standard error criterion. The left vertical line represents the final 24 variables identified. LASSO regression showed that the variables (coefficients) related to the development of milestones in PD patients included age (0.024246), pFN (-0.017086), UA (-0.001545), Alb (-0.068079), and hsCRP (0.016296). Abbreviations: Alb= albumin, hsCRP= hypersensitive C-reactive protein, pFN=plasma fibronectin, UA= uric acid.

Supplementary Figure 3. The determination of cutoff values for each risk factor with enumeration method.



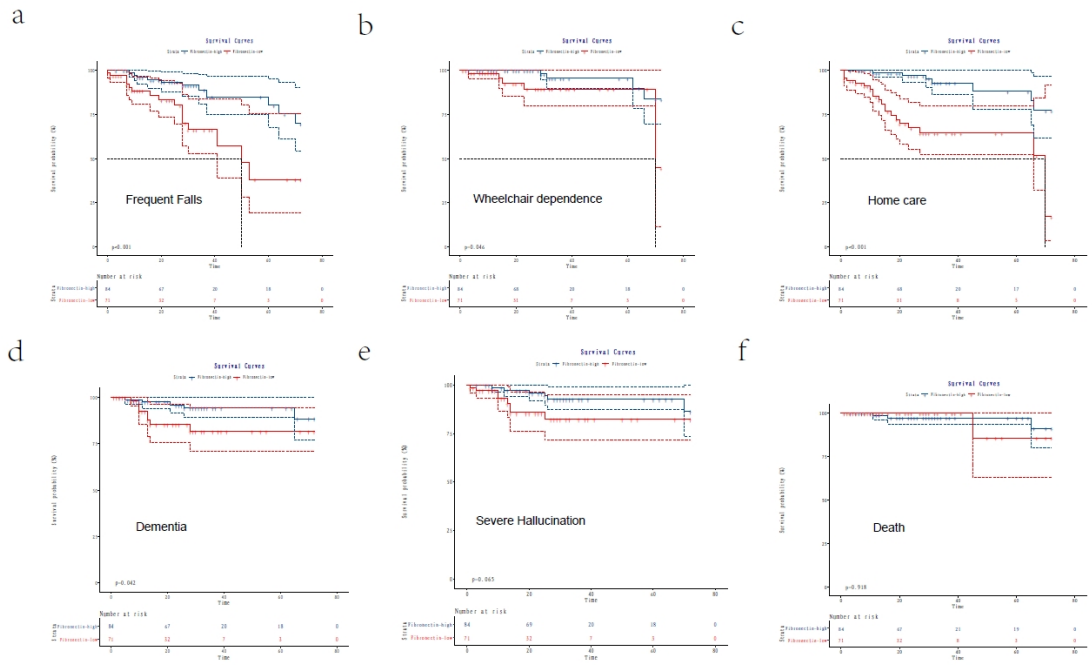
The cutoff values of the predictive factors were as follows: (A) age is 54 years at onset, (B) Alb is 37.8 g, (C) pFN is 197.6 mg/L, (D) hsCRP is 2.1 mg/L and (E) UA, is 306 μmol/L, respectively. Abbreviations: Alb= albumin, hsCRP= hypersensitive C-reactive protein, pFN=plasma fibronectin, UA= uric acid.

Supplementary Figure 4. The AUC values for each risk factor.



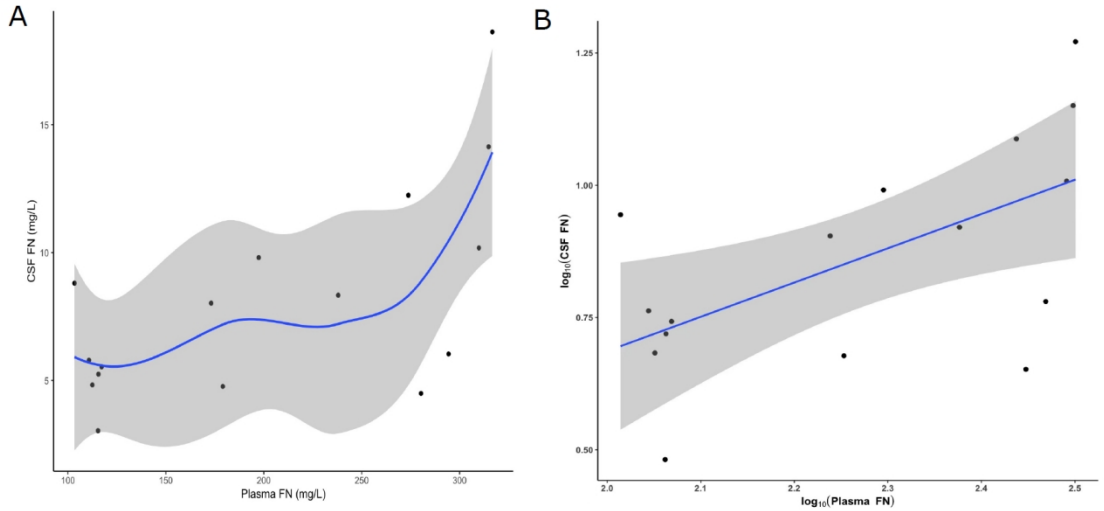
(A-E), The AUC values of the predictive factors. Abbreviations: ROC= Receiver Operating Characteristic Curve; AUC= Area Under Curve.

Supplementary Figure 5. Predicting disease progression by baseline pFN as assessed by survival analysis



Survival curve for low-and high-pFN group were analysed. The disease progression was defined as the development of frequent falls (p<0.001) **(A)**, wheelchair dependence (p=0.046) (**B**), homecare (p<0.001) **(C)**, dementia (p=0.042) **(D)**, severe hallucination (p=0.065) **(E)** and death (p=0.918) **(F)**, respectively. Abbreviations: pFN=plasma fibronectin.

Supplementary Figure 6. CSF and plasma FN levels are highly correlated.



A, Significant associations were found between CSF FN and plasma FN levels in PD (n = 16, Spearman correlation coefficient = 0.547, p = 0.028). The shaded areas represent the 99% credible intervals around the model estimates. **B,** Log $_{10}$ transformation of FN in CSF and plasma was positively linearly correlated after adjusting for age, sex and disease course (F = 3.65, R^2 = 0.57, adjusted R^2 = 0.41, p = 0.04). Abbreviations: CSF= Cerebrospinal Fluid; pFN=plasma fibronectin; PD= Parkinson's disease.

Supplementary table 1. The selection and definition of 24 candidate markers based on peripheral inflammatory mechanisms.

Candidate markers	Definition	Detection methods	Reagent supplier	Basis for selection and References
Clinical variables				
Age (y)	Age at onset	/	/	1. Kempster, P. A. et al. [1]
Sex	Biological sex	/	/	1. Carlisle, S. M. et al.[2] 2. Abraham, D. S. et al.[3]
Disease duration (y)	Years from symptoms onset and diagnosis to first visit	/	/	1. Riggeal, B. D.[4]
HBP	High blood pressure diagnosed by a physician according to the diagnostic criteria SBP ≥140mmHg and/ or DBP ≥90mmHg.	/	/	1. Ng, Y. F.[5] 2. Chen, J.[6] 3. Qiu, C.[7] 4. Simon, K. C.[8]
DM	Diabetes mellitus diagnosed by a physician according to the diagnostic criteria FPG ≥7mmol/L or 2h PG ≥11.1mmol/L or HbA1c ≥6.5%.	/	/	1. Zhong, Q.[9] 2 . Senkevich, K.[10] 3. Ou, R.[11] 4. de Pablo-Fernandez, E. [12] 5. Chohan, H.[13]
Inflammatory protein or cells				
Hs-CRP, mg/L	high-sensitivity C-reactive protein	CRP analyzer	HEALES, Shenzhen, China	1. Mehta, N.[14] 2. Gao, M. [15] 3. Sun, S. [16] 4. Shen, J. [17] 5. Lyra, P. [18] 6. Kim, R. [19] 7. Dommershuijsen, L. J.[20] 8. Yang, W. [21] 9. Mollenhauer, B. [22] 10. Song, I. U.[23]
Lymphocyte, G/L	Lymphocyte	Sysmex XN-10 Hematology	Sysmex Corporation, Kobe, Japan	1. Dommershuijsen, L. J.[20]

		Analyzer		
Neutrophils, G/L	Neutrophils	Sysmex XN-10 Hematology Analyzer	Sysmex Corporation, Kobe, Japan	1. Munoz-Delgado, L. [24] 2. Kim, R. [25] 3. Kara, S. P. [26] 4. Fan, Y. [27] 5. Vitte, J. [28]
L/N ratio,	L/N ratio	/	Sysmex Corporation, Kobe, Japan	1. Liu, Z. [29] 2. Kara, S. P.[26] 3. Munoz-Delgado, L. [24]
Metabolic inflammation molecular markers				
UA, μmol/L	Uric acid	Uric Acid (UA) Colorimetric Assay Kit (E-BC-K016-M)	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Mollenhauer, B. [22] 2. de Lau, L. M. [30] 3. Annanmaki, T. [31] 4. Schlesinger, I. [32] 5. Winquist, A. [33] 6. Gonzalez-Aramburu, I. [34] 7. Pellecchia, M. T. [35] 8. Koros, C. [36] 9. Bougea, A. [37] 10. Koros, C. [38] 11. Seifar, F. [39] 12. Koros, C. [40]
Chol, mmol/L	Cholesterol	Total Cholesterol and Cholesteryl Ester Fluorometric Assay Kit (E-BC-F032)	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Alrouji, M. [41] 2. Wang, K. [42] 3. Pingale, T. D. [43] 4. Garcia-Sanz, P. [44] 5. Yang, W. [21] 6. Jin, U. [45] 7. Simon, K. C. [8]
HDL-C, mmol/L	High-density lipoprotein cholesterol	High-density Lipoprotein Cholesterol (HDL-C) Colorimetric Assay Kit (Double	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Mollenhauer, B. [22] 2. Park, J. H. [46] 3. Liu, Z. [29] 4. Yang, W. [21] 5. Staszewski, J. [47] 6. Hottman, D. A. [48]

		reagents) (E-BC-K221-M)		
LDL-C, mmol/L	Low-density lipoprotein cholesterol	Low-density Lipoprotein Cholesterol (LDL-C) Colorimetric Assay Kit (Double reagents) (E-BC-K205-M)	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Wei, Q. [49]
Glu, mmol/L	Fasting glucose	Glucose (GLU) Fluorometric Assay Kit (E-BC-F037)	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Chohan, H. [13] 2. Foltynie, T. [50]
Alb, g/L	Albumin	Human ALB(Albumin) ELISA Kit (E-EL-H6105)	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Gao, M.[15] 2. Sun, S.[16] 3. Shen, J.[17]
A/G ratio	Albumin/Globulin ratio	Human IgG (Immunoglobulin G) ELISA Kit (E-EL-H0169c)	Elabscience Biotechnology (Wuhan, Hubei, China).	/
ADA, IU/L	Adenosine deaminase	Adenosine Deaminase (ADA) Activity Assay Kit (E-BC-K197-M)	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Huang, W. [51] 2. Ivanova, E. A. [52] 3. Chiba, S. [53] 4. Chiba, S. [54]
RBP, mg/L	Retinol binding protein	Human RBP4(Retinol Binding Protein 4) ELISA Kit (E-EL-H1581c)	Elabscience Biotechnology (Wuhan, Hubei, China).	Jimenez-Jimenez, F. J. [55]
SOD, kU/l	Superoxide dismutase	Total Superoxide Dismutase (T-SOD) Activity	Elabscience Biotechnology (Wuhan, Hubei, China).	1. Yang, W. [21]

		Assay Kit (Hydroxylamine Method) (E-BC-K019-S)		
LDH, IU/L	Lactate dehydrogenase	AU680 Chemistry Analyzer	Beckman Coulter Inc; Brea, California, U.S.A.	1. Chowdhury, C. S. [56] 2. Wu, X. B. [57] 3. Miyoshi, N. [58]
pFN, mg/L	Plasma fibronectin	Human Fibronectin ELISA kit (ab108848)	Abcam, Cambridge, UK	1. Yoshizaki, S. [59] 2. Lemanska-Perek, A. [60] 3. Dhanesha, N. [61] 4. Dhanesha, N. [62] 5. Khan, M. M. [63] 6. Brenmoehl, J. [64] 7. Goos, M. [65] 8. Bouvenot, G. [66] 9. Stecher, V. J. [67] 10. Scott, D. L. [68]
Hemostatic and coagulative molecular markers				
FIB, g/L	Fibrinogen	Clauss method by STA compact coagulometer	Diagnostica Stago, Asnieres, France	1. Torbitz, V. D.[69] 2. Wong, K. T.[70]
DDI, mg/L	D-dimer	Clauss method by STA compact coagulometer	Diagnostica Stago, Asnieres, France	1. Feng, J.
PLG, %	Plasminogen	Clauss method by STA compact coagulometer	Diagnostica Stago, Asnieres, France	1. Xu, Q. [71] 2. Reuland, C. J. [72] 3. Pan, H. [73]

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Supplementary table 2. The selection and definition of 6 milestones.

Milestones	Definition	Reference
Frequent Falling*	A notation in the clinical record, or a reporting from caregiver, that multiple falls due to their Parkinson's disease, were occurring. Isolated falls were ignored, even if fractures had resulted.	Hely, M. A, et al. Mov Disord,2005,20(2),190-9[3]. Peter A. Kempster. Brain, 2010: 133; 1755–1762[2].
Wheelchair dependence	After an optimal drug treatment, persistent losing a walking ability without wheelchair or walker	De Pablo-Fernandez, E, et al. JAMA Neurol, 2019,76(4): 470-479[4]
Residential care	Long-term admission to a residential high-level care facility; Or highly dependent of nursing staff at home.	Peter A. Kempster, et al. Brain, 2010: 133; 1755–1762[2]. De Pablo-Fernandez, E, et al. JAMA Neurol, 2019, 76(4): 470-479[4].
Dementia	A neuropsychological diagnosis of dementia was made on the basis of impairment in memory and at least two other areas of cognitive functioning; If no neuropsychological assessment was made, a diagnosis of dementia was based on a Clinical Dementia Rating ≥1 with supporting evidence from carers of gradual cognitive decline sufficient to impair daily function; If reports from caregivers, diagnosis of dementia was made on the basis of cognitive impairments severe enough to significantly affect tasks of daily.	Hely, Mariese A, et al. Mov Disord. 2008, 30; 23(6): 837-44.[5] De Pablo-Fernandez, E. JAMA Neurol, 2019,76(4): 470-479[4].
Severe hallucinations*	Reporting of persistent formed visual hallucinations; hallucination related to infection, dehydration and antiparkinsonian agents were excluded.	Poewe, W. Pract, et al. Neurol, 8(4): 238-41[6]. Peter A. Kempster, Brain, 2010: 133; 1755–1762[2].
Death	Death results from the aggravation of Parkinson's disease or Parkinson's related complications. Death caused by other accidental events were excluded.	De Pablo-Fernandez, E, et al. JAMA Neurol, 2019,76(4):470-479[4].

*The time of onset of each of these was recorded. The first recording of visual hallucinations or falling was taken as the time of onset.

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Supplementary table 3. Survival estimates of subjects by 1-year, 3-year, and 5-year probability generated by nomogram.

Abbreviations: HR, hazard ratio; NA, non available.

1- year survival probability	Number of subjects	Median survival (months)	HR (95% CI)	3- year survival probability	Number of subjects	Median survival (months)	HR (95% CI)	5-year survival probability	Number of subjects	Median survival (months)	HR (95% CI)
≤0.6	9	9	5-13	≤0.1	2	2	2-2	≤0.1	11	9	5-13
≥0.9	72	N/A	N/A	≥0.5	176	66	55-77	≥0.5	100	NA	NA
0.6-0.7	12	16	13-19	0.1-0.2	4	5.5	1-10	0.1-0.2	13	16	10-22
0.7-0.8	35	41	28-54	0.2-0.3	7	22	3-41	0.2-0.3	23	64	28-100
0.8-0.9	90	55	47-63	0.3-0.4	10	16	13-19	0.3-0.4	35	32	19-45
				0.4-0.5	19	64	NA	0.4-0.5	36	70	36-104

Supplementary Table 4. Baseline characteristic in HC and PD cohorts.

Characteristic	**HC (n=218)**	**PD (n=218)**	***P* Value**
Demographic			
Median age at onset, y	61(53-68)	63 (54-68)	0.162
Sex, No. (%)			
Male	129 (59.18%)	129 (59.18%)	1.000
Baseline laboratory test			
Hs-CRP, mg/L	0 (0-0.88)	0.5 (0-0.82)	<0.001***
UA, μmol/L	334.56 (266.75-398.75)	316.00 (262.75-378.25)	0.177
Alb, g/L	40.30 (38.20-42.10)	39.80 (37.50-42.00)	0.387
pFN, mg/L	212.50 (190.75-239.00)	200.12 (182.70-227.0)	<0.001***

Abbreviations: Alb, albumin; Hs-CRP, hypersensitive C-reactive protein; pFN, plasma fibronectin; UA, uric acid.
***p<0.001.

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Supplementary table 5. Multivariate analysis (crude and adjusted) of predictors selected by LASSO regression procedure in the discovery cohort (all).

	Definition	Univariate Cox regression		Multivariate Cox regression	
		Crude HR	P Value	Adjusted HR	P Value
First milestone					
pFN	Continuous, mg/L	0.981 (0.973-0.989)	<0.001***	0.982 (0.974-0.99)	<0.001***
Age	Continuous, years	1.023 (1.001-1.045)	0.040	1.014 (0.993-1.036)	0.198
UA	Continuous, μmol/L	0.997 (0.994-0.999)	0.019	0.998 (0.995-1)	0.096
Alb	Continuous, g/L	0.912 (0.852-0.977)	0.009**	0.954 (0.889-1.024)	0.194
Hs-CRP	Continuous, mg/L	1.037 (1.014-1.060)	0.001**	1.019 (0.995-1.044)	0.115
Fall					
pFN	Continuous, mg/L	0.974 (0.964-0.984)	<0.001***	0.974 (0.964-0.985)	<0.001***
Age	Continuous, years	1.028 (0.999-1.058)	0.057	1.024 (0.993-1.055)	0.127
UA	Continuous, μmol/L	0.994 (0.990-0.998)	0.002**	0.995 (0.991-0.999)	0.006
Alb	Continuous, g/L	0.936 (0.856-1.023)	0.143	0.965 (0.877-1.061)	0.459
Hs-CRP	Continuous, mg/L	1.024 (0.979-1.071)	0.294	1.007 (0.965-1.051)	0.758
Wheelchair					
pFN	Continuous, mg/L	0.982 (0.968-0.997)	0.019*	0.982 (0.966-0.998)	0.024*
Age	Continuous, years	1.026 (0.985-1.068)	0.224	1.024 (0.981-1.068)	0.274
UA	Continuous, μmol/L	0.999 (0.994-1.004)	0.686	1.000 (0.995-1.005)	0.877
Alb	Continuous, g/L	0.986 (0.864-1.127)	0.841	1.035 (0.905-1.185)	0.613
Hs-CRP	Continuous, mg/L	1.050 (0.999-1.104)	0.055	1.049 (0.997-1.104)	0.068
Homecare					
pFN	Continuous, mg/L	0.989 (0.977-1.001)	0.061	0.989 (0.977-1.002)	0.091
Age	Continuous, years	1.021 (0.987-1.056)	0.232	1.016 (0.982-1.052)	0.359
UA	Continuous,	1.000 (0.995-1.004)	0.824	1.000 (0.996-1.004)	0.965

	μmol/L				
Alb	Continuous, g/L	0.954 (0.854-1.065)	0.401	0.987 (0.879-1.108)	0.824
Hs-CRP	Continuous, mg/L	1.034 (0.993-1.077)	0.105	1.028 (0.986-1.072)	0.197
Dementia					
pFN	Continuous, mg/L	0.989 (0.975-1.004)	0.149	0.992 (0.976-1.007)	0.279
Age	Continuous, years	1.034 (0.988-1.082)	0.238	1.028 (0.982-1.077)	0.238
UA	Continuous, μmol/L	0.997 (0.992-1.003)	0.322	0.998 (0.993-1.003)	0.480
Alb	Continuous, g/L	0.928 (0.812-1.061)	0.273	0.943 (0.816-1.090)	0.428
Hs-CRP	Continuous, mg/L	0.997 (0.896-1.109)	0.957	0.985 (0.874-1.111)	0.804
Hallucination					
pFN	Continuous, mg/L	0.986 (0.970-1.003)	0.101	0.988 (0.972-1.005)	0.165
Age	Continuous, years	1.003 (0.955-1.054)	0.892	0.994 (0.946-1.045)	0.810
UA	Continuous, μmol/L	0.995 (0.988-1.002)	0.160	0.996 (0.990-1.003)	0.281
Alb	Continuous, g/L	0.933 (0.800-1.087)	0.373	0.956 (0.808-1.130)	0.597
Hs-CRP	Continuous, mg/L	1.011 (0.921-1.111)	0.814	1.003 (0.911-1.104)	0.953
Death					
pFN	Continuous, mg/L	0.998 (0.970-1.026)	0.871	1.001 (0.969-1.033)	0.971
Age	Continuous, years	1.024 (0.939-1.117)	0.588	0.967 (0.866-1.081)	0.559
UA	Continuous, μmol/L	0.995 (0.983-1.006)	0.350	1.000 (0.988-1.013)	0.963
Alb	Continuous, g/L	0.516 (0.352-0.756)	0.001	0.549 (0.339-0.889)	0.015
Hs-CRP	Continuous, mg/L	1.084 (1.035-1.134)	0.001	1.041 (0.987-1.099)	0.142

Abbreviation: Alb, albumin; HR, hazard ratio; Hs-CRP, hypersensitive C-reactive protein; pFN, plasma fibronectin; UA, uric acid; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

Supplementary table 6. Baseline Characteristic between high and low pFN in DCE cohort.

Characteristic	**Validation cohort (n=84)**	**DCE cohort (n=67)**		***P* Value**	
		Low pFN (n=33)	**High pFN (n=34)**	**Validation vs. DCE**	**Low vs. High pFN**
Male No. (%)	52 (61.90%)	25 (75.76%)	18(52.94%)	0.866	0.075
Age	64 (58.5-69.5)	66 (61-71)	63 (56.8-68.2)	0.875	0.191
Disease duration	3 (2-6)	3 (1.5-6.5)	4 (2-7)	0.910	0.404

Abbreviation: DCE; pFN, plasma fibronectin; UA, uric acid.

人磷酸化α-突触核蛋白(p-α-SYN)酶联免疫

检测试剂盒使用说明书

AE90954Hu

使用前仔细阅读本说明书。本酶联免疫试剂盒是基于双抗体夹心技术原理，来检测人磷酸化α-突触核蛋白(p-α-SYN)，只能用于研究用途，不得用于医学诊断。

用　　途：用于人血清、血浆及相关液体样本中磷酸化α-突触核蛋白(p-α-SYN)测定。

工作原理

本试剂盒采用的是生物素双抗体夹心酶联免疫吸附法（ELISA）测定样品中人磷酸化α-突触核蛋白(p-α-SYN)水平。向预先包被了人磷酸化α-突触核蛋白(p-α-SYN)单克隆抗体的酶标孔中加入人磷酸化α-突触核蛋白(p-α-SYN)，温育；温育后，加入生物素标记的抗 p-α-SYN 抗体。再与链霉亲和素-HRP 结合，形成免疫复合物，再经过温育和洗涤，去除未结合的酶，然后加入底物 A、B，产生蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅与样品中人磷酸化α-突触核蛋白(p-α-SYN)的浓度呈正相关。

试剂盒组成

试剂盒组成	48 孔配置	96 孔配置	保存
说明书	1 份	1 份	
封板膜	2 片（48）	2 片（96）	
密封袋	1 个	1 个	
酶标包被板	1×48	1×96	2-8℃保存
标准品 120ng/L	0.5ml×1 瓶	0.5ml×1 瓶	2-8℃保存
标准品稀释液	3ml×1 瓶	6ml×1 瓶	2-8℃保存
链霉亲和素-HRP	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
生物素标记的抗 p-α-SYN 抗体	0.5ml×1 瓶	1 ml×1 瓶	2-8℃保存
显色剂 A 液	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
显色剂 B 液	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
终止液	3ml×1 瓶	6ml×1 瓶	2-8℃保存
浓缩洗涤液	（20ml×20 倍）×1 瓶	（20ml×30 倍）×1 瓶	2-8℃保存

需要而未提供的试剂和器材

1. 37℃恒温箱。
2. 标准规格酶标仪。
3. 精密移液器及一次性吸头
4. 蒸馏水，
5. 一次性试管
6. 吸水纸

注意事项

1. 从 2-8℃取出的试剂盒，在开启试剂盒之前要室温平衡至少 30 分钟。酶标包被板开封后如未用完，板条应装入密封袋中保存。
2. 各步加样均应使用加样器，并经常校对其准确性，以避免试验误差
3. 严格按照说明书的操作进行，试验结果判定必须以酶标仪读数为准.
4. 为避免交叉污染，要避免重复使用手中的吸头和封板膜。
5. 不用的其它试剂应包装好或盖好。不同批号的试剂不要混用。保质前使用。
6. 底物 B 对光敏感，避免长时间暴露于光下。

洗板方法

手工洗板方法：甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍几次；将稀释后的洗涤液至少 0.35ml 注入孔内，浸泡 1-2 分钟。根据需要，重复此过程数次。

自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中

标本要求

1．不能检测含 NaN3 的样品，因 NaN3 抑制辣根过氧化物酶的（HRP）活性。

2．标本采集后尽早进行提取，提取按相关文献进行，提取后应尽快进行实验。若不能马上进行试验，可将标本放于-20℃保存，但应避免反复冻融。

操作程序

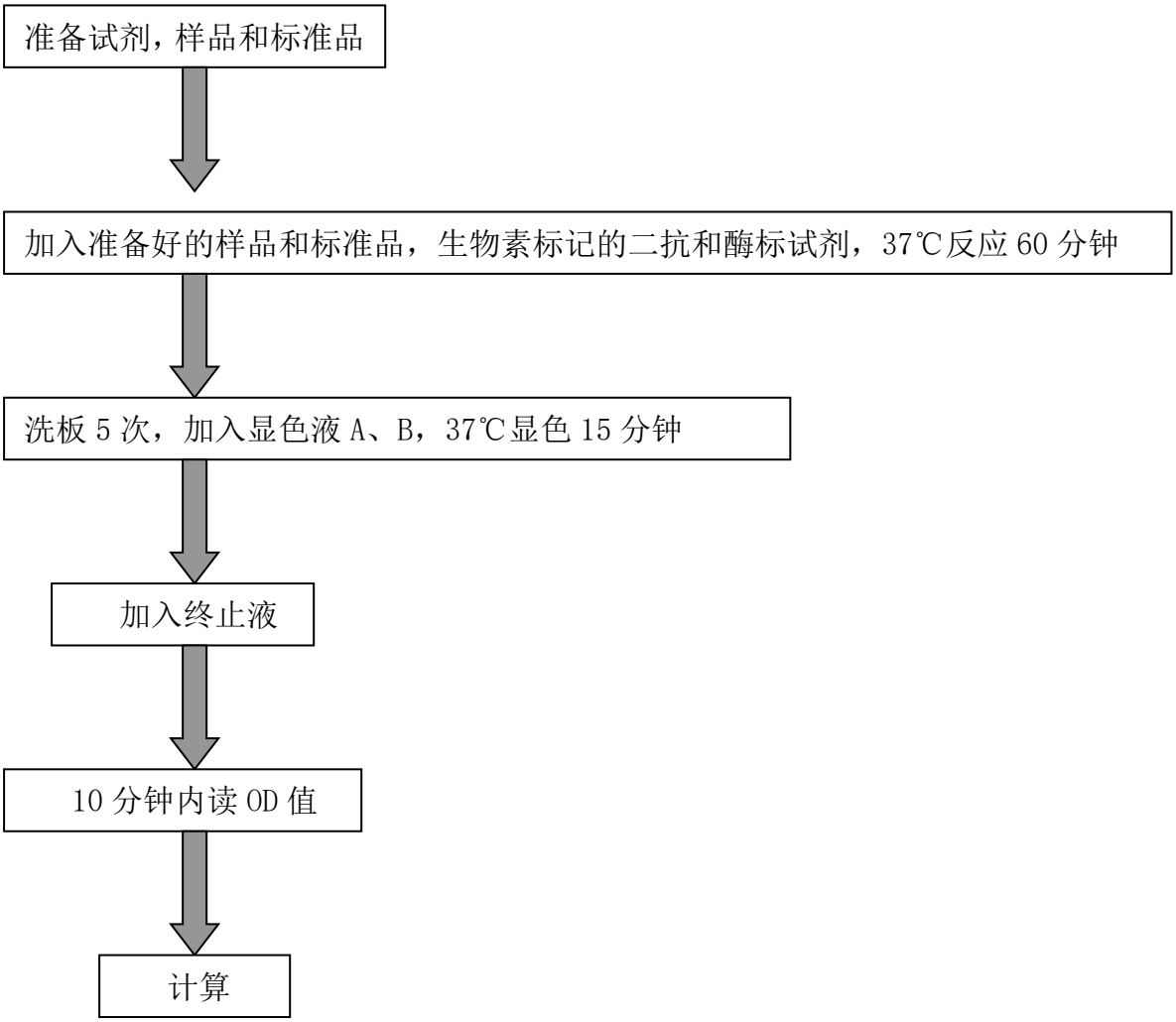
1. 标准品的稀释：（本试剂盒提供原倍标准品一支，用户可按照下列图表在小试管中进行稀释。）

60ng/L	5 号标准品	120μl 的原倍标准品加入 120μl 标准品稀释液
30ng/L	4 号标准品	120μl 的 5 号标准品加入 120μl 标准品稀释液
15ng/L	3 号标准品	120μl 的 4 号标准品加入 120μl 标准品稀释液
7.5ng/L	2 号标准品	120μl 的 3 号标准品加入 120μl 标准品稀释液
3.75ng/L	1 号标准品	120μl 的 2 号标准品加入 120μl 标准品稀释液

2. 根据待测样品数量加上标准品的数量决定所需的板条数。每个标准品和空白孔建议做复孔。每个样品根据自己的数量来定，能使用复孔的尽量做复孔。
3. 加样：1）空白孔，**空白对照孔不加样品，生物素标记的抗 p-α-SYN 抗体，链霉亲和素-HRP，只加显色剂 A&B 和终止液，其余各步操作相同**；2）标准品孔：加入**标准品 50ul，链霉亲和素-HRP50ul(标准品中已事先整合好生物素抗体，故不加)**；3）代测样品孔：加入**样本 40ul**，然后各加入**抗 p-α-SYN 抗体 10ul、链霉亲和素-HRP50ul**，盖上封板膜，轻轻震荡混匀，37℃温育 60 分钟。
4. 配液：将 30 倍浓缩洗涤液用蒸馏水 30 倍稀释后备用。
5. 洗涤：小心揭掉封板膜，弃去液体，甩干，每孔加满洗涤液，静置 30 秒后弃去，如此重复 5 次，拍干。
6. 显色：每孔先加入显色剂 A50ul，再加入显色剂 B50μl，轻轻震荡混匀，37℃避光显色 15 分钟.
7. 终止：每孔加终止液 50μl，终止反应（此时蓝色立转黄色）。
8. 测定：以空白孔调零，450nm 波长依序测量各孔的吸光度（OD 值）。 测定应在加终止液后 10 分钟以内进行。
9. 根据标准品的浓度及对应的 OD 值计算出标准曲线的直线回归方程，再根据

样品的 OD 值在回归方程上计算出对应的样品浓度。也可以使用各种应用软件来计算。

操作程序总结：



试剂盒性能：

1.样品线性回归与预期浓度相关系数 R 值为 0.92 以上。
2.批内与批间应分别小于 9%和 15%

检测范围：

检测范围： 1.5ng/L -60ng/L

保存条件及有效期：

保存：2-8℃。
有效期：6 个月(2-8℃)。

Human p-α-SYN ELISA Kit

Instruction

This kit is only for scientific research, and shall not be used as a clinical diagnosis of use.

Purpose

This kit allows for the determination of p-α-SYN concentrations in Human serum, cell culture supernatant, and other biological fluids.

Principle

The kit assay Human p-α-SYN level in the sample， add Human p-α-SYN antibody to microtiter plate wells, after Incubating,add Biotinylated anti–p-α-SYN -antibody , then Combined Streptavidin-HRP, become complex, after Incubating and washing Completely, Add TMB substrate solution,TMB substrate becomes blue color, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of p-α-SYN in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials provided with the kit

Materials provided with the kit	48determinations	96 determinations	Storage
User manual	1	1	
Closure plate membrane	2	2	
Sealed bags	1	1	
Microelisa stripplate	1	1	2-8℃
Standard：120ng/L	0.5ml×1 bottle	0.5ml×1 bottle	2-8℃
Standard diluent	3ml×1 bottle	6ml×1 bottle	2-8℃
Streptavidin-HRP	3ml×1 bottle	6ml×1 bottle	2-8℃
Biotinylated anti –p-α	0.5ml×1 bottle	1ml×1 bottle	2-8℃

-SYN -antibody			
Chromogen Solution A	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution B	3ml×1 bottle	6ml×1 bottle	2-8℃
Stop Solution	3ml×1 bottle	6ml×1 bottle	2-8℃
wash solution	（20ml×20 fold）×1bottle	（20ml×30 fold）×1bottle	2-8℃

Materials required but not supplied

1. 37 ℃ incubator
2. Standard microplate reader(450nm)
3. Precision pipettes and Disposable pipette tips.
4. deionized water.
5. Disposable Test tube

6 Absorbent paper

Important notes

1. The kit takes out from the refrigeration environment should be balanced 15-30 minutes in the room temperature, ELISA plates coated if has not use up after opened, the plate should be stored in Sealed bag.
2. add Sample with sampler Each step, And proofread its accuracy frequently, avoids the experimental error.
3. Please according to use instruction strictly, The test result determination must take the microtiter plate reader as a standard.
4. Use new disposal plastic pipette tips and Closure plate membrane for each standard, in order to avoid cross contamination.
5. Do not mix reagents with those from other lots.
6. The substrate evade the light preservation.

Specimen requirements

1. extract as soon as possible after Specimen collection,and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can’t, specimen can be kept in -20 ℃ to preserve, Avoid repeated freeze-thaw cycles.

2. Can’t detect the sample which contain NaN3, because NaN3 inhibits HRP active.

Assay procedure

1. Dilute and add sample:Dilute Original density Standard as follow table:

60ng/L	5 Standard	120μl Original density Standard+120μl Standard diluent
30ng/L	4 Standard	120μl 5 Standard+120μl Standard diluent
15ng/L	3 Standard	120μl 4 Standard+120μl Standard diluent
7.5ng/L	2 Standard	120μl 3 Standard +120μl Standard diluent
3.75ng/L	1 Standard	120μl 2 Standard +120μl Standard diluent

2. according testing Sample numbers to define how many wells need, Standard and blank suggested Do holes.

3.add sample：1) blank wells: (blank comparison wells don’t add sample , Biotinylated anti –p-α-SYN -antibody and Streptavidin-HRP ,other each step operation is same); 2) Standard wells: add Standard 50μl and Streptavidin-HRP 50μl; 3) testing Sample wells: add sample 40μl,then add anti –p-α-SYN -antibody 10μl , Streptavidin-HRP 50μl. closing plate with Closure plate membrane ,incubate for 60 min at 37℃.

4.Configurate liquid: 30-fold(or 20-fold) wash solution diluted 30-fold (or 20-fold) with distilled water and reserve.

5.washing：Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.

6.color：Add Chromogen Solution A 50ul and Chromogen Solution B to each well, evade the light preservation for 15 min at 37℃

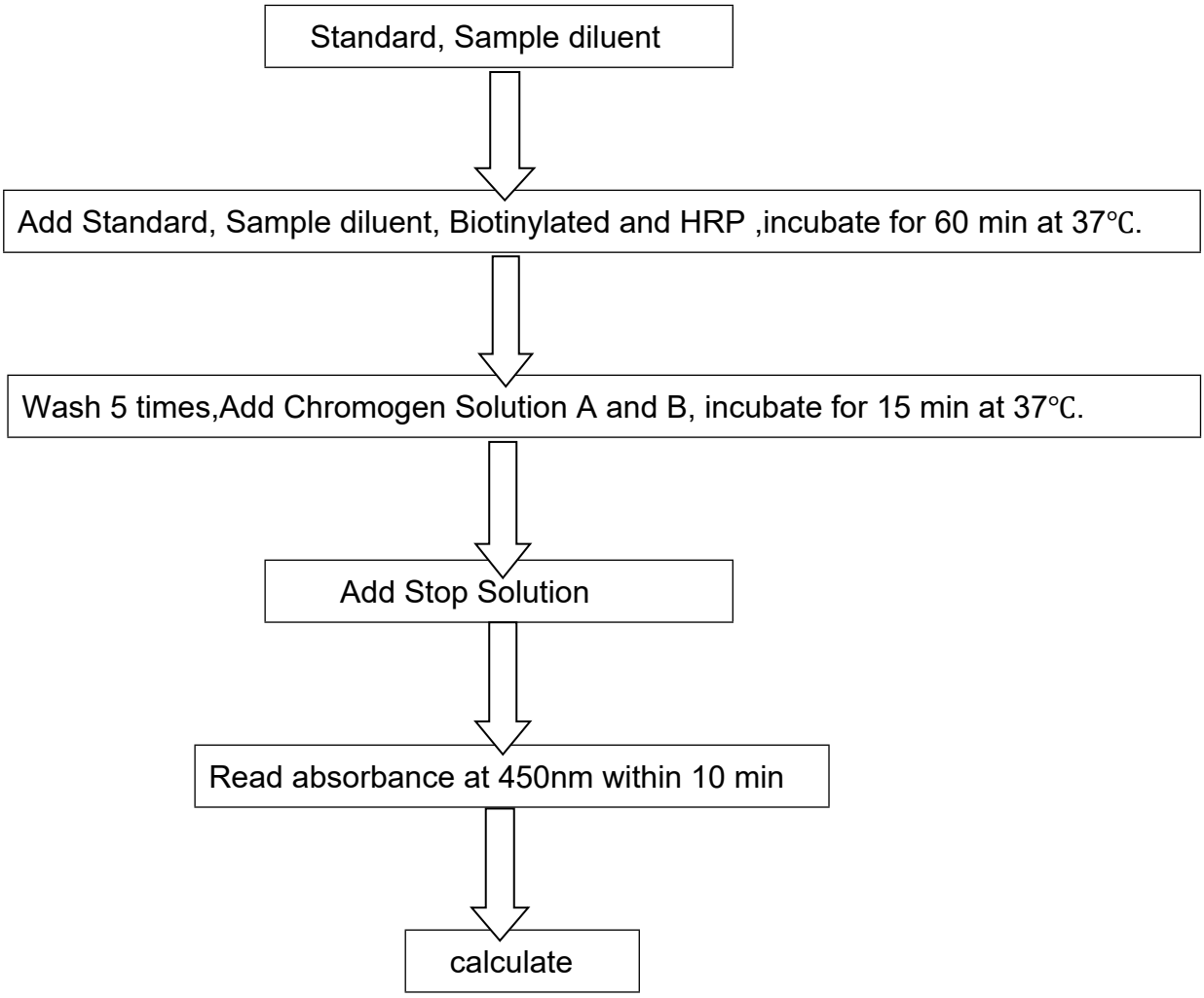
7.Stop the reaction：Add Stop Solution50μl to each well, Stop the reaction(the blue color change to yellow color).

8.assay：take blank well as zero , Read absorbance at 450nm after Adding

Stop Solution and within 10min.

9. Calculate of result

Steps description



Assay range

1.5ng/L -60ng/L

Storage and validity

1. Storage： 2-8℃.
2. validity： six months.