COSMO BIO CO., LTD.

α - Synuclein Aggregation Assay Kit

Cat. No. CSR-SYN01-COS

Updated on May 24th, 2016

※ This product is manufactured under license from Takashi Nonaka, Ph.D. and Masato Hasegawa, Ph.D. of Department of Dementia and Higher Brain Function,Tokyo Metropolitan Institute of Medical Science.

【 I 】Introduction

Dementia is a disease that causes memory and judgement loss, there is no fundamental treatment developed for it. Years of scientific research has proven that protein aggregation occurs in the degeneration part of the brain for many neurodegenerative diseases including dementia, indicating appearance of this aggregation is closely related to these diseases development and progression.

Aggregation occurs when normal protein forms abnormal structure for particular reasons and accumulates in the cell, which each disease has different aggregating protein. It is known that protein called " α -Synuclein" forms abnormal structure in Parkinson Disease / Lewy Body Dementia / Multiple system atrophy patient brain.

α -Synuclein Aggregation Assay Kit mimics intracellular aggregation of " α -Synuclein", which allows in vitro screening of active ingredients.

【II -1】Kit Components

Storage：4℃　Do not freeze

- α -Synuclein Aggregation Assay Kit can be used up to 300 wells (24 well plate size) when prepared as described in【III-2】(page 2).
- Plasmid vectors of α -Synuclein Aggregation Assay Kit are generated by DNA2.0 (www.dna20.com/).

Components	Volume	Quantity	Precautions
pCMV-SNCA (α - Synuclein Expression plasmid vector, Red cap)	32 µL (conc. 1.25 µg/µL)	1 vial	Use appropriate protective equipment (such as gloves and glasses) when handling. (The purchaser should not transfer and/or resell copies and/or derivatives of this product to any third parties.)
pCMV-NC(Negative control vector, Green cap)	5 µL (conc. 1.25 µg/µL)	1 vial	
pCMV-dGFP(dGFP Expression plasmid vector, Blue cap)	5 µL (conc. 1.25 µg/µL)	1 vial	
20 mM Tris-HCl Buffer (pH7.4)	10 mL	1 bottle	Use appropriate protective equipment (such as gloves and glasses) when handling. Keep in a cool dark place once unpacked.
F- α Syn (α - Synuclein Fibrils, Yellow cap)	32 µL (conc. 1 µg/µL)	1 vial	Prior to disposing F- α Syn, please autoclave (134℃ , 20 min.), or add SDS (>3%) prior to autoclave (121℃ , 20min.). If above condition cannot be matched, please dispose accordingly to your local rules and regulations.
Multifectam (Gene transfection reagent)	0.33 mg	1 bottle	Keep at 4℃ or -20℃ .

【II -2】Required but not provided:

- SH-SY5Y cell line
- Culture medium (recommended: DE/F-12, 10% FBS, 1% NEAA)
- Opti-MEM® or Serum-Free medium (e.g. ThermoFisher Scientific, cat. no. 31985062)
- DNase, RNase-Free sterile purified water

* Please perform steps III -1 through III -3 under germless condition in clean bench.

【III -1】Preparation of working solution

- pCMV-SNCA (α - Synuclein Expression plasmid vector, Red cap), pCMV-NC（Negative control vector, Green cap）, pCMV-dGFP（dGFP Expression plasmid vector, Blue cap）

  Please adjust concentration with sterile purified water based on your amount needs (just before use).
  e.g. 1uL/well of 10 fold dilution when used for 24 well plate size.

- F- α Syn (α - Synuclein Fibrils, Yellow cap)

  Please adjust concentration with sterile purified water based on your amount needs (just before use).

  e.g. 1uL/well of 10 fold dilution when used for 24 well plate size.

  * CAUTION: Prior to disposing F- α Syn, please autoclave (134 ℃ , 20 min.), or add SDS (>3%) prior to autoclave (121℃ , 20min.). If above condition cannot be matched, please dispose accordingly to your local rules and regulations.

- MultiFectam (Gene transfection reagent)

  Add 1mL of sterile purified water (room temp.) to MultiFectam and let it sit still for c.a. 10sec., vortex thoroughly. Reagent can be used up to 3 months (4 ℃) or 6 months (-20 ℃) following reconstitution. Performance of MultiFectam after reconstitution is guaranteed up to 6 times of freeze-thaw cycle; to prevent it below 6 times or less, please aliquot reconstituted reagent into multiple vials.

【III -2】How to use the kit

- Below example is for use of SH-SY5Y cell line.
- For the 1st try out or when using other cell lines, please perform preliminary experiment by adjusting usage amount of pCMV-dGFP plasmids based on below protocol.

1 **Culture necessary cell amount for preliminary experiment.**

2 **Plate 3 × 10^4 cells/cm^2 into each multi-well plate.**

3 **When cells becomes confluent (typically 1-3 days depends on cell line and culture medium conditions), follow methods in below【III-3】to adjust pCMV-SNCA and F- α Syn concentration to add into medium.**

4 **Incubate 4 hours, replace with culture medium.**

5 **α – Synuclein aggregation & precipitation will occur in 1-3 days. Follow methods in below【IV】to detect this level.**

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【Ⅲ -3】Preparation example of pCMV-SNCA / F- α Syn / MultiFectam

● Below example is for preparation of 1 well of 24 well plate size (culture medium 0.5 mL/well).

1 **Combine reagents according to the table below.**

	Transfection test	Example
10 fold diluted pCMV-SNCA(if negative control: pCMV-NC)	-	23 µL
10 fold diluted F- α Syn	-	1 µL(0.125 µg/ well)
pCMV-dGFP	0.05 ～ 0.5 µg/well	1 µL (0.1 µg/ well)
20 mM Tris-HCl Buffer（pH 7.4）	up to 25 µL	23 µL(up to 25 µL)
Total 25 µL	25 µL	25 µL

*** When confirming transfection efficiency by pCMV-dGFP expression, adjust plasmid amount to become 0.05 ～ 0.5 µg/well. Replace F- α Syn (1 µL) with 20 mM Tris-HCl Buffer（pH 7.4）. Excitation/fluorescent wavelength of dGFP is 510/521 nm.**

2 **Add 3 µL MultiFectam, mix the reaction by vortexing to ensure that all reagents are thoroughly mixed. After mixing, spin down and incubate at room temperature for 30 min. (pCMV-SNCA / F- α Syn / MultiFectam complex will form.)**

3 **Add 22 µL Opti-MEM® or Serum-Free medium, mix the reaction by vortexing to ensure that all reagents are thoroughly mixed. After mixing, spin down and incubate at room temperature for 5 min (still standing).**

4 **Aliquot 50 µL of pCMV-SNCA / F- α Syn / MultiFectam complex mix into each well, shake gently to distribute evenly. If toxicity is observed, either a.) adjust addition amount to each well, b.) adjust mixture ratio & addition amount of MultiFectam and pCMV-SNCA.**

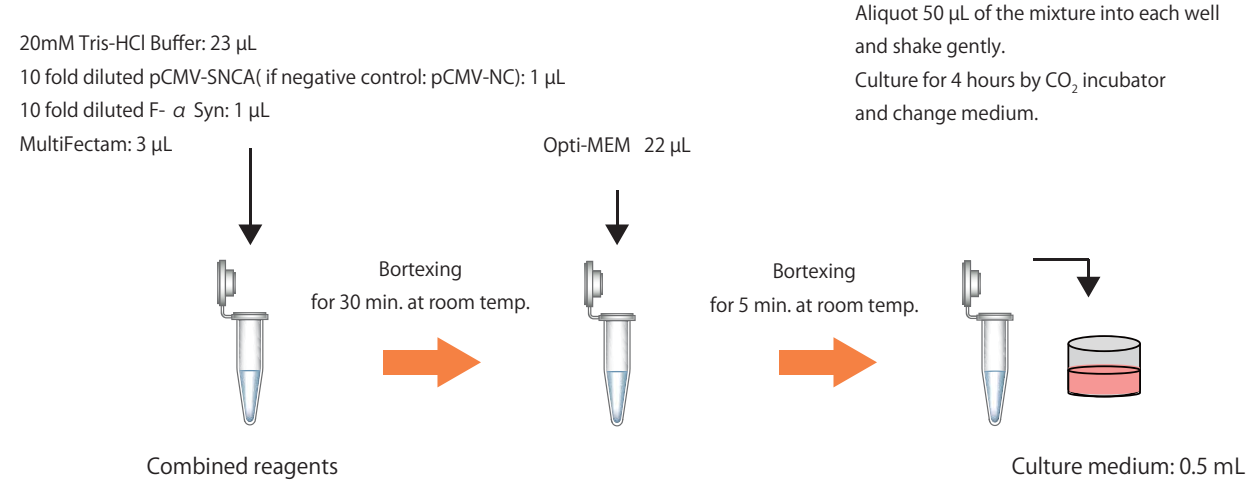

Fig. 1: Preperation of pCMV-SNCA / F- α Syn / MultiFectam（For 1 well of 24-well plate size）

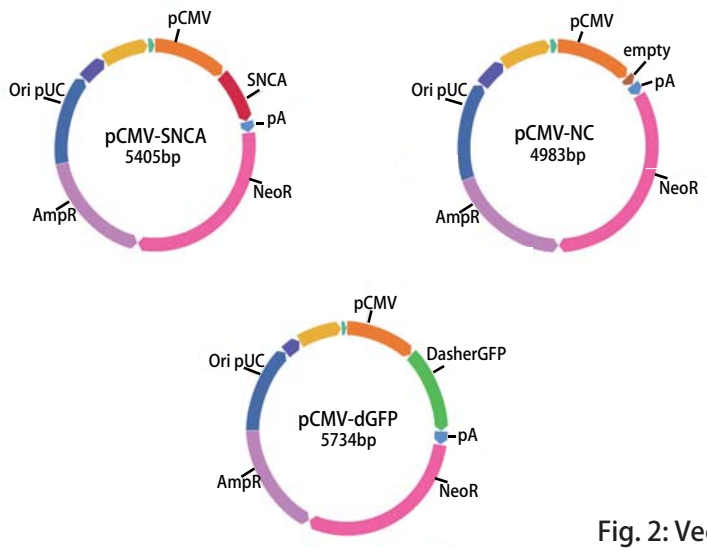

Fig. 2: Vector map

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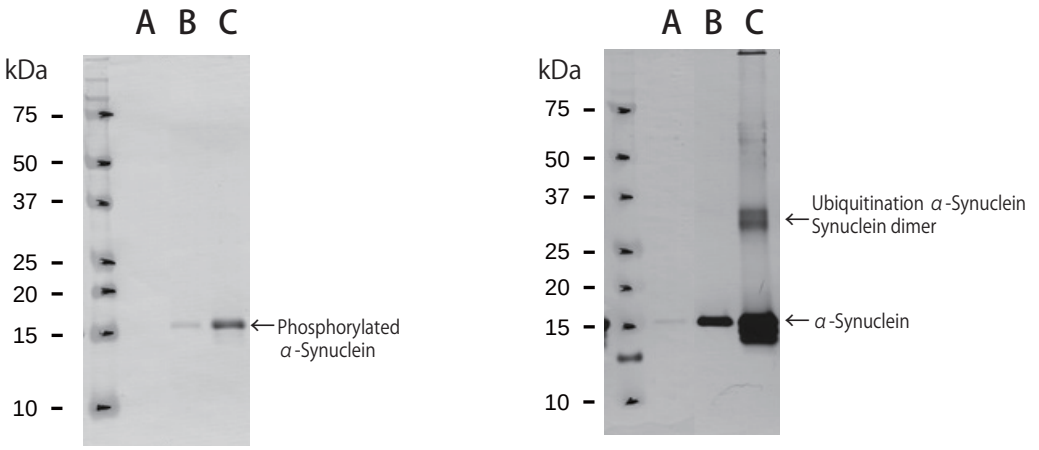
【IV】Detection example of α -Synuclein aggregation complex

1. Western blotting (Use of various synuclein antibodies)

Experimental example:

1. Remove culture medium.
2. Add 1 mL PBS to each well, collect cells by pipetting.
3. Centrifuge (4,500 rpm [1,800 x g], 5 min., room temp.), collect pellet.
4. Add 0.1 mL Lysis Buffer (10 mM Tris-HCl, pH 7.5 containing 0.8 M NaCl, 1 mM ethyleneglycol bis(2-aminoethyl ether)-N,N,N,N-tetraacetic acid (EGTA), 1 mM DTT and 1% N-Lauroylsarcosine sodium salt), sonicate mixture.
5. Centrifuge (50,000 rpm [100,000 x g], 20 min., room temp.).
6. Add 40 µL 2 x SDS Buffer (0.125 M Tris-HCl pH6.8, 200 mM 1,4 dithiothreitol (DTT), 4% sodium dodecyl sulfate(SDS) , 10% Sucrose, 0.01% BPB) to pellet, sonicate mixture, heat (100 ℃, 5 min), apply 10 µL/well for western blotting.



Anti Phosphorylated α-Synuclein, Monoclonal Antibody (pSyn#64) 015-25191 (Wako)

Anti α-Synuclein (131-140) (Rabbit) CAC:TIP-SN-P09 (Cosmo Bio)

A. pCMV-NC (Negative control vector)
B. pCMV-SNCA (α - Synuclein Expression plasmid vector)
C. pCMV-SNCA + F- α Syn (α - Synuclein Expression plasmid vector + α - Synuclein Fibrils)

Fig. 3: Experimental example of detecting synuclein aggregation complex by western blotting

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2. Amyloid structure fluorescent staining kit

By use of Amyloid Structure Fluorescent Staining Kit (Cosmo Bio, cat. no. CSR-SYN02-COS), dual staining of aggregated α -Synuclein and nucleolus can be performed.

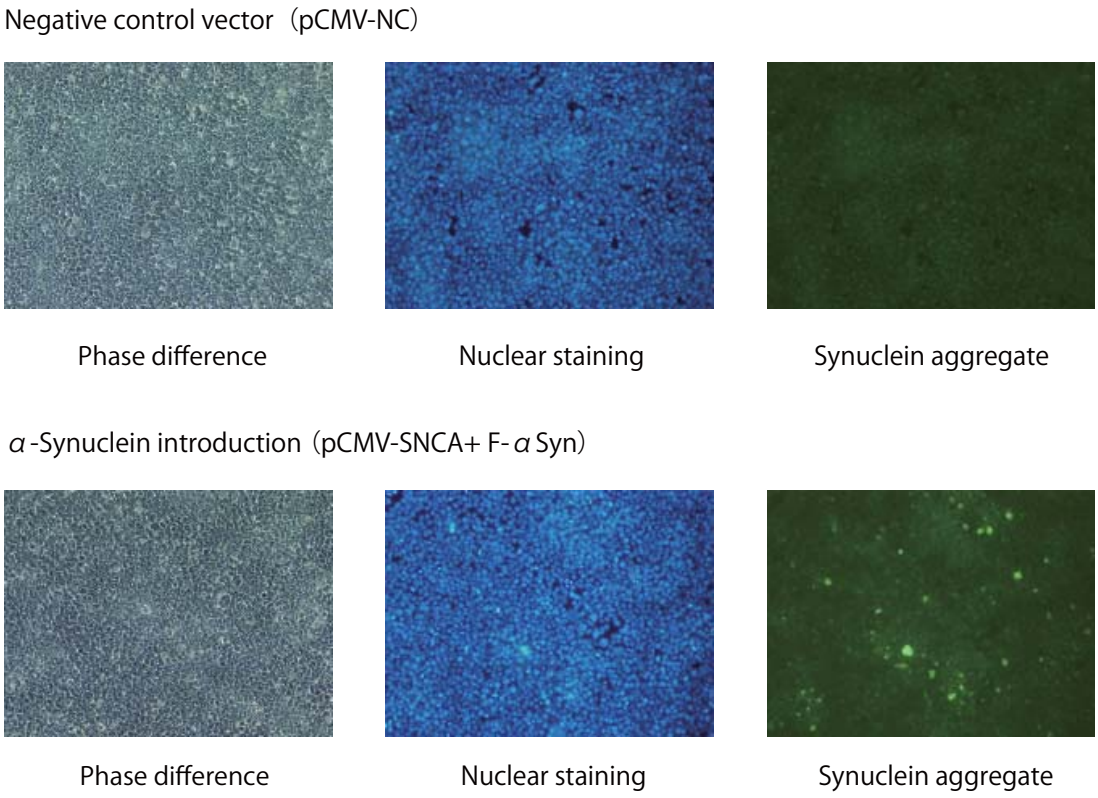

Fig. 4: Example of detection using Amyloid Structure Fluorescent Staining Kit

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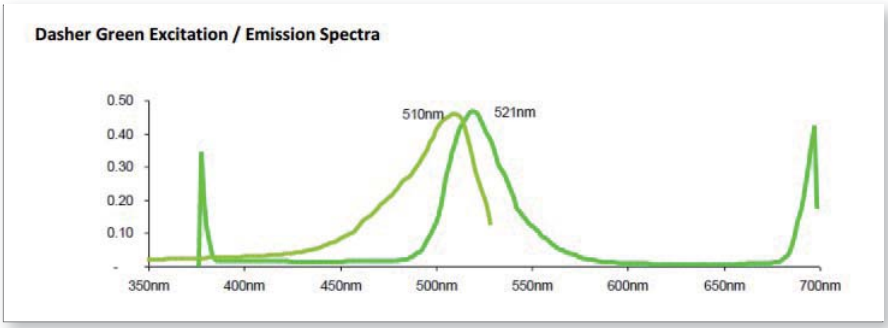
【V】Trouble shooting

1. When α -synuclein aggregation doesn't express, use the cells which have high efficiency for gene transfer or which have plenty of satisfactory results. To inspect transfer efficiency, Control Vvector (pCMV-dGFP) is in this kit. Refer to【Ⅲ - 2】for how to use. For Excitation/Emission Spectra, please see the data shown below.
2. When you change the amount of consumption , please try to increase usage of pCMV-SNCA / F- α Syn / MultiFectam complex mix about 2.5 times or more without changing the mixing ratio of this manual mentioned to【Ⅲ -3】.
3. If the detection does not work well, we recommend that you do both of the staining and western blotting. When you can't detect by both, please try【V】-1.



【Ⅵ】Reference

[1] J Biol Chem. 2010 Nov 5;285(45):34885-98. doi: 10.1074/jbc.M110.148460. Epub 2010 Aug 30.
Seeded aggregation and toxicity of {alpha}-synuclein and tau: cellular models of neurodegenerative diseases.
Nonaka T, Watanabe ST, Iwatsubo T, Hasegawa M.
PMID : 20805224

【Ⅶ】Related products

Description	Cat. No.	Quantity
Amyloid Fluorescent Staining Kit	CSR-SYN02-COS	100 TEST
α -Synuclein Fibrils	CSR-SYN03-COS	0.1 MG

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