



# ALPHA-SYNUCLEIN TOTAL KIT STANDARD

## PROTOCOL

**Part #** 6FNSYCDA

**Amount:** 150 ng

**Concentration:** 500 ng/mL

**Form:** Lyophilized

**Store at:** 2-8°C before reconstitution

**Revision:** 01 - March 2019



**For research use only. Not for use in diagnostic procedures.**

### APPLICATIONS

The Alpha-synuclein total kit standard must be used to perform the absolute quantification of cellular total alpha-synuclein extracted with Cisbio's lysis buffer.

Our technical support team can help you to set up this protocol or another one.

Please contact us at [www.cisbio.com/contact-us](http://www.cisbio.com/contact-us)

### REAGENT PREPARATION

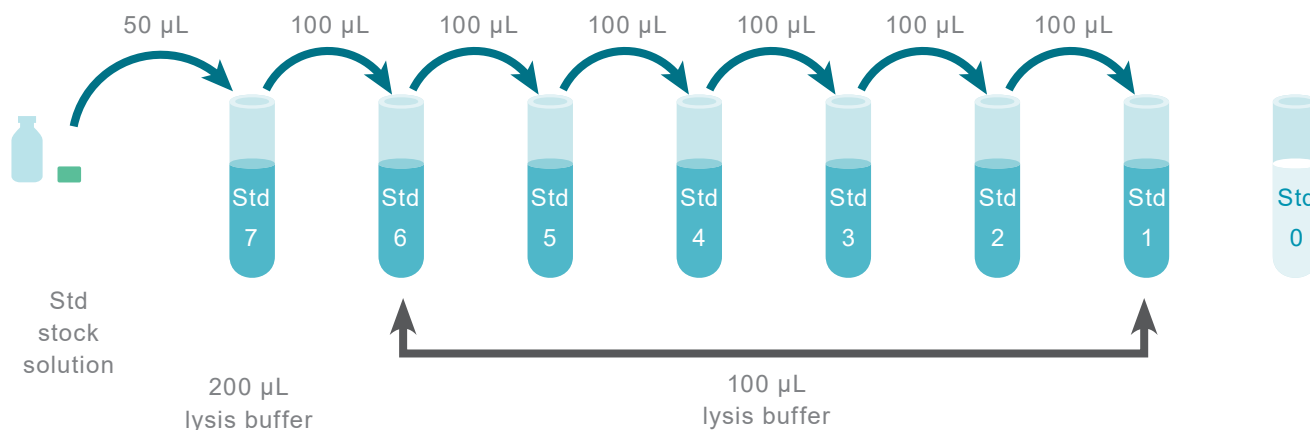
#### TO PREPARE WORKING STANDARD SOLUTIONS:

- Reconstitute the standard vial following the instructions indicated on the vial label.
- Each well requires 16  $\mu$ L of standard.
- Dilute the standard stock solution serially with the lysis buffer used to prepare your samples.
- To preserve the stability of the total alpha-synuclein Standard, more than one freeze/thaw should be avoided, and aliquots should be made.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

► **A recommended standard dilution procedure is listed and illustrated below:**

- Dilute the standard stock solution 5-fold with the appropriate lysis buffer to prepare high standard (Std 7 = 100 ng/mL):  
Take 50  $\mu$ L of standard stock solution and add it to 200  $\mu$ L of lysis buffer. Mix gently.
- Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:  
Dispense 100  $\mu$ L of lysis buffer into each vial for Std 6 to Std 0.  
Add 100  $\mu$ L of standard to 100  $\mu$ L of lysis buffer, mix gently, and repeat the 1/2 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.  
This will create 7 standards for the analyte. Std 0 (Negative control) is lysis buffer alone.

STANDARD	SERIAL DILUTIONS	TOTAL ALPHA-SYNUCLEIN (ng/mL) WORKING SOLUTION
Standard stock solution	Reconstitute following label instructions.	500
Standard 7	50 $\mu$ L standard stock solution + 200 $\mu$ L lysis buffer	100
Standard 6	100 $\mu$ L Standard 7 + 100 $\mu$ L lysis buffer	50
Standard 5	100 $\mu$ L Standard 6 + 100 $\mu$ L lysis buffer	25
Standard 4	100 $\mu$ L Standard 5 + 100 $\mu$ L lysis buffer	12.5
Standard 3	100 $\mu$ L Standard 4 + 100 $\mu$ L lysis buffer	6.25
Standard 2	100 $\mu$ L Standard 3 + 100 $\mu$ L lysis buffer	3.13
Standard 1	100 $\mu$ L Standard 2 + 100 $\mu$ L lysis buffer	1.56
Standard 0	100 $\mu$ L lysis buffer	0



## DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit [www.cisbio.com/htrf-ratio-and-data-reduction](http://www.cisbio.com/htrf-ratio-and-data-reduction)

## RESULTS

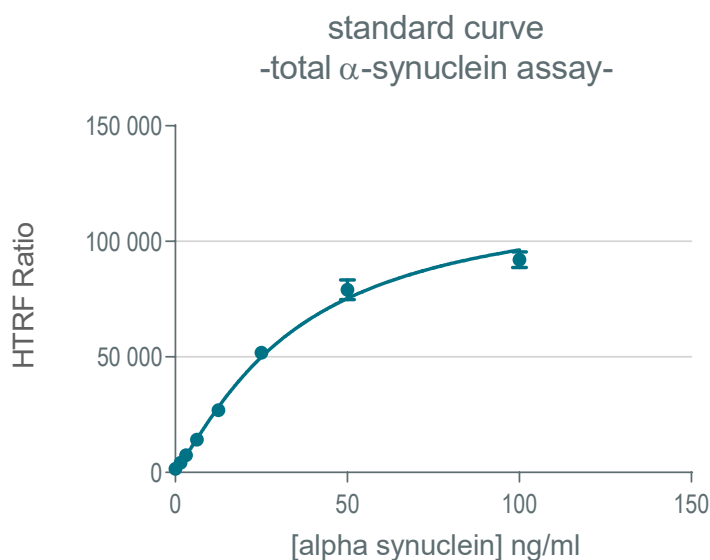
The following data must not be substituted for the data obtained in the laboratory, and should be considered only as an example (readouts on PHERAstar FS with a flash lamp). Results may vary from one HTRF® compatible reader to another.

The data below were obtained using the reagents of the Alpha-synuclein total kit (# 6FNSYPEG, 6FNSYPEH)

Standard curve fitting with the 4 Parameter Logistic (4PL 1/y2)\* model

\* For more information about curve fitting, please visit <http://www.cisbio.com/4PL-regression>

	Alpha-Synuclein (ng/mL)	Ratio (1)	CV (2)
Std 0 - negative control	0	1550	7%
Std 1	1.57	4153	2%
Std 2	3.13	7451	1%
Std 3	6.25	14131	3%
Std 4	12.5	26923	1%
Std 5	25	51765	2%
Std 6	50	79071	5%
Std 7	100	92053	4%



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