MANUAL

Technology: HTRF® Pathway readout

Tau Total Kit standard

Part number	**64NTAUCDA**
Concentration	90 000 pg/mL
Form	Lyophilized

Storage: 2-8°C before reconstitution

Amount : 27 000 pg

Version: 02 **Date:** February 2024

The Tau Total kit- standard must be used to perform the absolute quantification of cellular total Tau extracted with Revvity's lysis buffer.

REAGENT PREPARATION

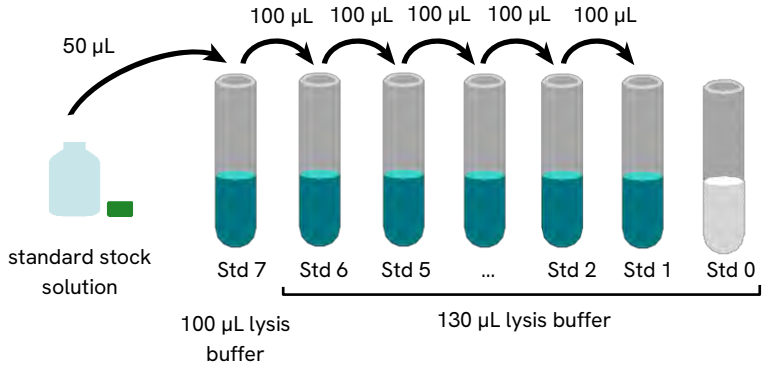
To prepare working standard solutions:

- Reconstitute the standard vial following the instructions indicated on the vial label.
- Each well requires 16 µL of standard.
- Dilute the standard stock solution serially with the lysis buffer used to prepare your samples.
- To preserve the stability of the total Tau 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 3-fold with the appropriate lysis buffer to prepare high standard (Std 7 = 30 000 pg/mL):
  Take 50 µL of standard stock solution and add it to 100 µL of lysis buffer. Mix gently.
- Use the high standard (Std 7) to prepare the standard curve using 1/2.3 serial dilutions as follows:
  Dispense 130 µL of lysis buffer into each vial for Std 6 to Std 0.
  Add 100 µL of standard to 130 µLof lysis buffer, mix gently, and repeat the 1/2.3 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	PREPARATION	WORKING SOLUTIONS ng/mL
Standard stock solution	Reconstitute following label instructions.	90 000
Standard 7	50 µL standard stock solution + 100 µL lysis buffer	30 000
Standard 6	100 µL Standard 7 + 130 µL lysis buffer	13 043
Standard 5	100 µL Standard 6 + 130 µL lysis buffer	5 671
Standard 4	100 µL Standard 5 + 130 µL lysis buffer	2 466
Standard 3	100 µL Standard 4 + 130 µL lysis buffer	1 072
Standard 2	100 µL Standard 3 + 130 µL lysis buffer	466.00
Standard 1	100 µL Standard 2 + 130 µL lysis buffer	203.00
Standard 0	130 µL lysis buffer	0

DATA REDUCTION AND 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.

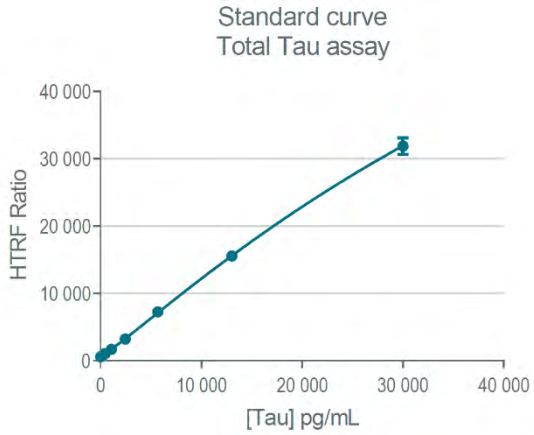
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit our website.

RESULTS

The following data must not be substituted for the data obtained in the laboratory, and should be considered only as an example. Results may vary from one HTRF® compatible reader to another.
The data below were obtained using the reagents of the Tau Total kit (# 64NTAUPEG, 64NTAUPEH)
Standard curve fitting with the 4 Parameter Logistic (4PL 1/y2)* model
* For more information about curve fitting, please visit our website.

	Tau (pg/mL)	Ratio (1)	%CV (2)
Std 0 Negative control	0	591	7.3
Std 1	203	785	5.3
Std 2	466	1 039	2.9
Std 3	1 072	1 669	4.5
Std 4	2 466	3 209	2.0
Std 5	5 671	7 262	2.0
Std 6	13 043	15 552	4.0
Std 7	30 000	31 882	3.9



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handling and storage. The use of the cell line will be done with appropriate safety and handling precautions to minimize health and envvvironmental impact.

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