MANUAL

ALPHA-TUBULIN HOUSEKEEPING STANDARD

Part # 64ATUBCDA
Amount: 3 µg
Concentration: 30 µg/mL
Form: Frozen
Store at: -60°C or below
Revision: #02 of September 2023

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

APPLICATIONS

The Alpha-Tubulin housekeeping standard must be used to perform the absolute quantification of cellular alpha-tubulin extracted with Revvity's lysis buffer.

Our technical support team can help you to set up this manual or another one.
Please contact us at www.revvity.com

REAGENT PREPARATION

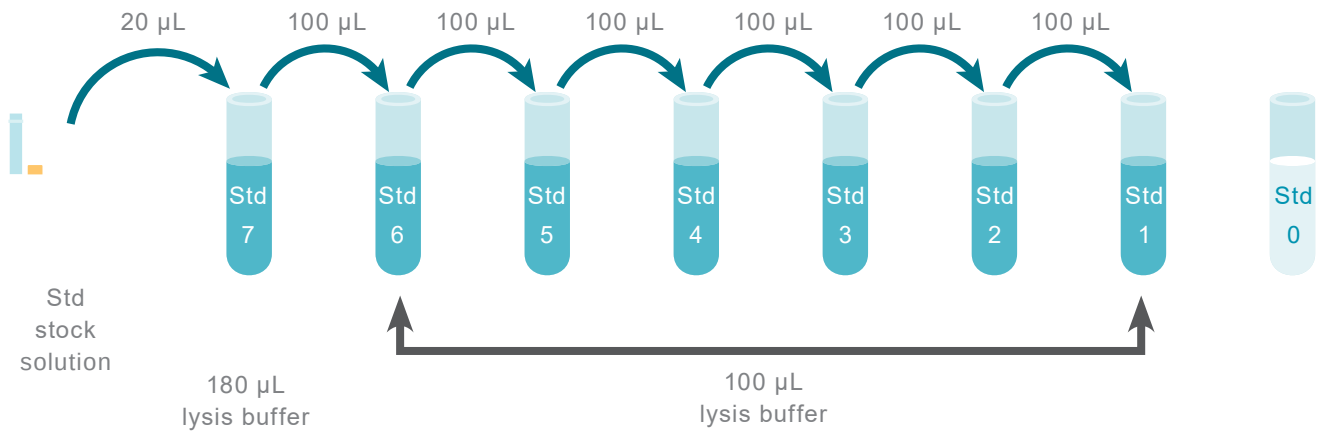
TO PREPARE WORKING STANDARD SOLUTIONS:

- The Alpha-Tubulin Housekeeping assay is compatible with the Revvity Phospho-total protein lysis buffers #1, #2, #3, and #4 provided with each phospho- or total protein assay kit. Therefore, the assay can be carried out on cell lysates generated with each of these lysis buffers (supplemented with the blocking reagent).
- Each well requires 4 µL of standard.
- Dilute the standard stock solution serially with the lysis buffer used to prepare your samples.
- To preserve the stability of the alpha-tubulin 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 10-fold with the appropriate lysis buffer to prepare high standard (Std 7 = 3 000 ng/mL):
  Take 20 µL of standard stock solution and add it to 180 µL 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 µL of lysis buffer into each vial for Std 6 to Std 0.
  Add 100 µL of standard to 100 µL 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	ALPHA-TUBULIN (ng/mL) WORKING SOLUTION
Standard stock solution	Thawed stock solution	30 000
Standard 7	20 µL standard stock solution + 180 µL lysis buffer	3 000
Standard 6	100 µL Standard 7 + 100 µL lysis buffer	1 500
Standard 5	100 µL Standard 6 + 100 µL lysis buffer	750
Standard 4	100 µL Standard 5 + 100 µL lysis buffer	375
Standard 3	100 µL Standard 4 + 100 µL lysis buffer	187.5
Standard 2	100 µL Standard 3 + 100 µL lysis buffer	93.8
Standard 1	100 µL Standard 2 + 100 µL lysis buffer	46.9
Standard 0	100 µL 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.

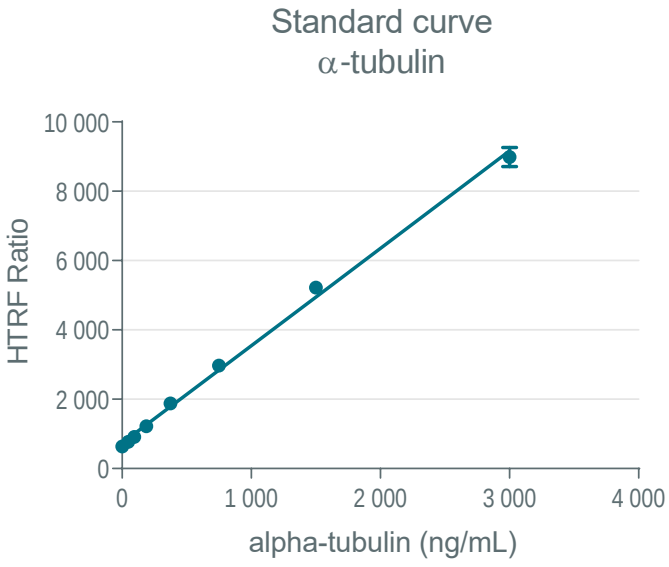
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

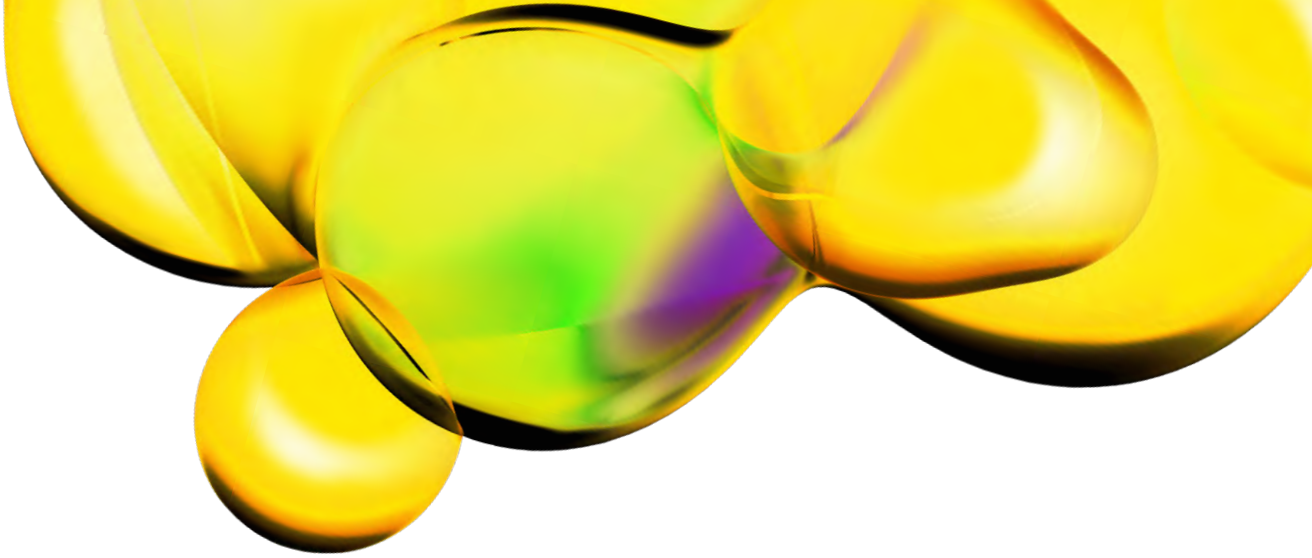
For more information about data reduction, please visit www.revvity.com

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-Tubulin Housekeeping kit (# 64ATUBPEG, 64ATUBPEH)

	Alpha tubulin (ng / mL)	Ratio (1)	CV (2)
Std 0 - negative control	0	638	1.1%
Std 1	46.875	771	1.2%
Std 2	93.75	913	1.9%
Std 3	187.5	1218	1.5%
Std 4	375	1876	4.7%
Std 5	750	2973	3.5%
Std 6	1 500	5220	3.0%
Std 7	3 000	8987	3.1%





Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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