MANUAL

Technology: HTRF® Protein-Protein Interaction

HTRF PD1 / PDL1 standard

Part number	**64PD1CDA**
Concentration	2.5 µM
Form	Frozen

Storage: ≤-60°C

Assay volume : 50 µL

Version: 02 **Date:** February 2024

Human PD1 /PDL1 standard is intended for use with the human PD1 /PDL1 binding kit. It provides a way to quantify the inhibition of the PD1 /PDL1 interaction.

REAGENT PREPARATION

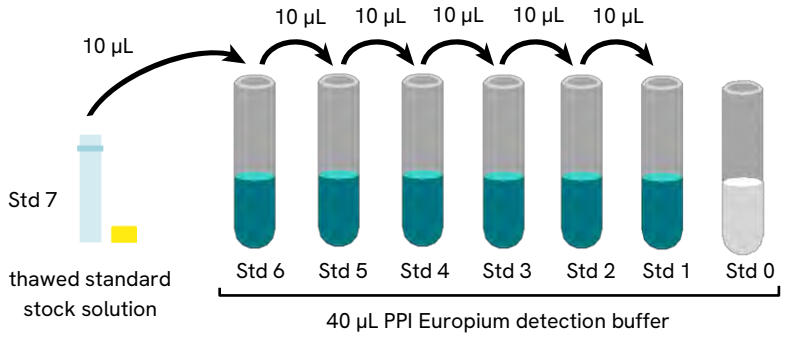
To prepare working standard solutions:

- Each well requires 2 µL of standard.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

- Thaw the PD1 / PDL1 standard stock solution, this yields the high standard (Std 7) = 2.5 µM (2 500 000 pM).
- Use the high standard (Std 7) to prepare the standard curve using 5-fold serial dilutions as follows:
    - Dispense 40 µL of PPI Europium detection buffer into each vial from Std 6 to Std 0
    - Add 10 µL of standard to 40 µL of PPI Europium detection buffer, mix gently and repeat the serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 is PPI Europium detection buffer alone.



STANDARD	PREPARATION	WORKING SOLUTIONS	FINAL CONCENTRATIONS
Standard 7 Standard stock solution	Thaw the PD1 / PDL1 standard stock solution	2.5 µM (2 500 000 pM)	250 000 pM
Standard 6	10 µL standard 7 + 40 µL PPI Europium detection buffer	500 000 pM	50 000 pM
Standard 5	10 µL standard 6 + 40 µL PPI Europium detection buffer	100 000 pM	10 000 pM
Standard 4	10 µL standard 5 + 40 µL PPI Europium detection buffer	20 000 pM	2 000 pM
Standard 3	10 µL standard 4 + 40 µL PPI Europium detection buffer	4 000 pM	400 pM
Standard 2	10 µL standard 3 + 40 µL PPI Europium detection buffer	800 pM	80 pM
Standard 1	10 µL standard 2 + 40 µL PPI Europium detection buffer	160 pM	16 pM
Standard 0	40 µL PPI Europium detection buffer	0 pM	0 pM

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.

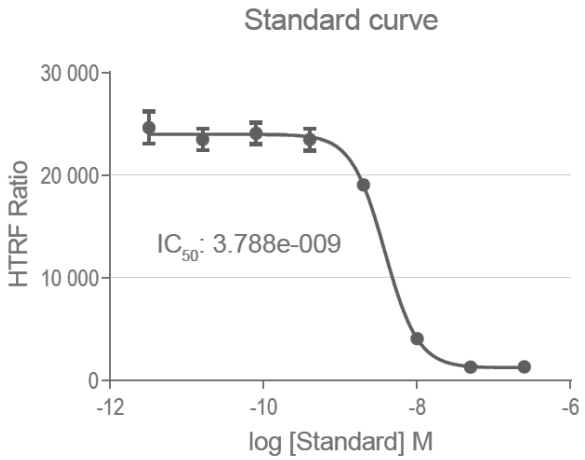
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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 (readouts on an HTRF compatible reader with a flash lamp). Results may vary from one HTRF® compatible reader to another.

The data below were obtained using the reagents of the PD1 / PDL1 binding assay kit - Ref# 64PD1PEG and #64PD1PEH



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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.

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

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