

# MANUAL

Technology: HTRF®

Protein-Protein Interaction

## HTRF PD1 / PDL1 standard

Part number	64PD1CDA
Concentration	2.5 $\mu$ M
Form	Frozen

Storage:  $\leq -60^{\circ}\text{C}$

Assay volume : 50  $\mu$ L

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

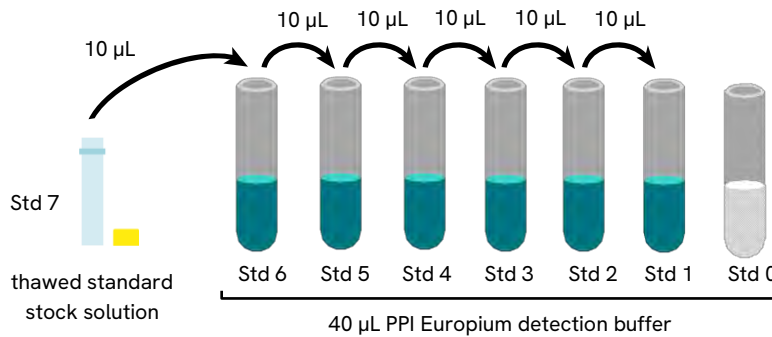
### To prepare working standard solutions:

- Each well requires 2  $\mu\text{L}$  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  $\mu\text{M}$  (2 500 000 pM).
- Use the high standard (Std 7) to prepare the standard curve using 5-fold serial dilutions as follows:
  - Dispense 40  $\mu\text{L}$  of PPI Europium detection buffer into each vial from Std 6 to Std 0
  - Add 10  $\mu\text{L}$  of standard to 40  $\mu\text{L}$  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 $\mu\text{M}$ (2 500 000 pM)	250 000 pM
Standard 6	10 $\mu\text{L}$ standard 7 + 40 $\mu\text{L}$ PPI Europium detection buffer	500 000 pM	50 000 pM
Standard 5	10 $\mu\text{L}$ standard 6 + 40 $\mu\text{L}$ PPI Europium detection buffer	100 000 pM	10 000 pM
Standard 4	10 $\mu\text{L}$ standard 5 + 40 $\mu\text{L}$ PPI Europium detection buffer	20 000 pM	2 000 pM
Standard 3	10 $\mu\text{L}$ standard 4 + 40 $\mu\text{L}$ PPI Europium detection buffer	4 000 pM	400 pM
Standard 2	10 $\mu\text{L}$ standard 3 + 40 $\mu\text{L}$ PPI Europium detection buffer	800 pM	80 pM
Standard 1	10 $\mu\text{L}$ standard 2 + 40 $\mu\text{L}$ PPI Europium detection buffer	160 pM	16 pM
Standard 0	40 $\mu\text{L}$ 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.

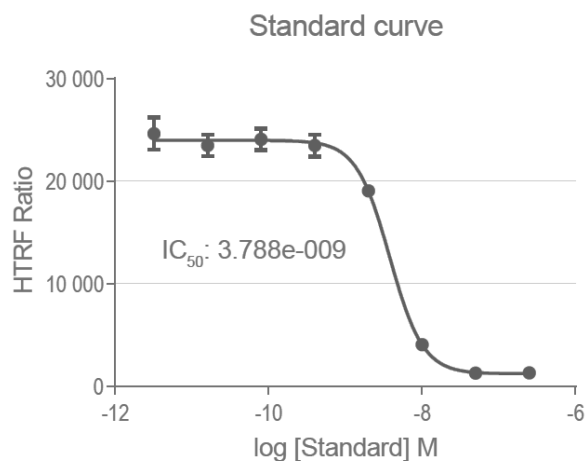
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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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