

MANUAL

Technology: HTRF® Protein-Protein Interaction

HTRF IL12/IL12RB1 standard

Part number	64BDIL12CDA
Concentration	5 μ M
Form	Frozen

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

Assay volume : 40 μ L

Version: 02

Date: February 2024

The IL12/IL12Rb1 standard is intended for use with the IL12/IL12Rb1 binding kit. It provides a way to control assay reproducibility.

REAGENT PREPARATION

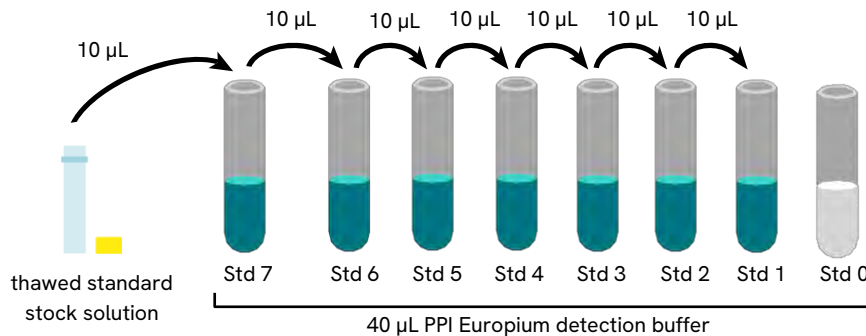
To prepare working standard solutions:

- 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 standard vial, the concentration of the IL12/IL12Rb1 standard stock solution = 5 μM (5 000 000 pM)
- Prepare the following dilutions:
 - Dilute the thawed standard stock solution 5-fold with PPI Europium detection buffer.
 In practice: take 10 μL of stock solution and add it to 40 μL of PPI Europium detection buffer. Mix gently. This yields the high standard (1 000 000 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 stock solution	Thaw the IL12/IL12Rb1 standard stock solution	5 μM (5 000 000 pM)	
Standard 7	10 μL standard stock solution + 40 μL PPI Europium detection buffer	1 000 000 pM	100 000 pM
Standard 6	10 μL standard 7 + 40 μL PPI Europium detection buffer	200 000 pM	20 000 pM
Standard 5	10 μL standard 6 + 40 μL PPI Europium detection buffer	40 000 pM	4 000 pM
Standard 4	10 μL standard 5 + 40 μL PPI Europium detection buffer	8 000 pM	800 pM
Standard 3	10 μL standard 4 + 40 μL PPI Europium detection buffer	1 600 pM	160 pM
Standard 2	10 μL standard 3 + 40 μL PPI Europium detection buffer	320 pM	32 pM
Standard 1	10 μL standard 2 + 40 μL PPI Europium detection buffer	64 pM	6.4 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.

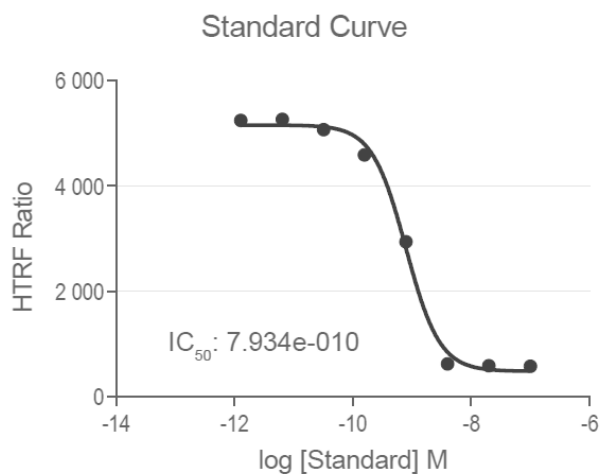
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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 HTRF® compatible reader). Results may vary from one HTRF® compatible reader to another.

The data below were obtained using the reagents of the IL12/IL12Rb1 binding assay kit - Ref#64BDIL12PEG and 64BDIL12PEH



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