

MANUAL

Technology: HTRF® Protein-Protein Interaction

HTRF IL17A/IL17RA standard

| Part number | 64BDIL17CDA | |
|---------------|-------------|--|
| Concentration | 2.5 μΜ | |
| Form | Frozen | |

Storage: ≤-60°C

Assay volume: 40 µL

Version: 02 **Date:** February 2024

The IL17A/IL17RA standard is intended for use with the IL17A/IL17RA binding kit. It provides a way to control assay reproductibility.

REAGENT PREPARATION

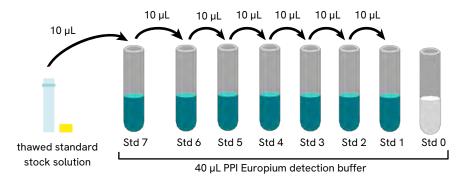
To prepare working standard solutions:

- 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 IL17A/IL17RA standard stock solution = $2.5 \mu M$ (2 500 000 pM)
- Prepare the following dilutions:
 - O 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 (500 000 pM).
 - o Use the high standard (Std 7) to prepare the standard curve using 5-fold serial dilutions as follows:
 - o Dispense 40 µL of PPI Europium detection buffer into each vial from Std 6 to Std 0
 - \circ 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 IL17A/IL17RA standard stock solution | 2.5 µM (2 500 000 pM) | |
| Standard 7 | 10 μL standard stock solution + 40 μL PPI Europium detection buffer | 500 000 pM | Mq 000 05 |
| Standard 6 | 10 μL standard 7 + 40 μL PPI Europium detection buffer | 100 000 pM | 10 000 pM |
| Standard 5 | 10 μL standard 6 + 40 μL PPI Europium detection buffer | 20 000 pM | 2 000 pM |
| Standard 4 | 10 μL standard 5 + 40 μL PPI Europium detection buffer | 4 000 pM | 400 pM |
| Standard 3 | 10 μL standard 4 + 40 μL PPI Europium detection buffer | 800 pM | 80 pM |
| Standard 2 | 10 μL standard 3 + 40 μL PPI Europium detection buffer | 160 pM | 16 pM |
| Standard 1 | 10 μL standard 2 + 40 μL PPI Europium detection buffer | 32 pM | 3.2 pM |
| Standard 0 | 40 uL PPI Europium detection buffer | Ma 0 | Ma 0 |

DATA REDUCTION AND INTERPRETATION

1) Calculate the ratio of the acceptor and donor emission signals for each individual well.

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.

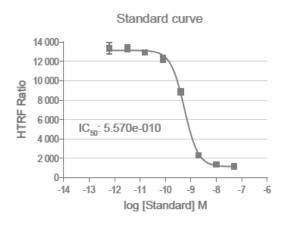
$$CV (\%) = \frac{Standard deviation}{Mean Ratio} \times 100$$

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 IL17A/IL17RA binding assay kit - Ref#64BDIL17PEG and 64BDIL17PEH



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