



SEROTONIN 5HT1A LABELED CELLS

Serotonin 5HT1A labeled cells for: 200 tests

Part#: C1TT15HT1A

Rev: #03 of October 2025

Store at: -80°C or below, see expiration date on package label.

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

ASSAY PRINCIPLE

The Tag-lite Serotonin 5HT1A cells transiently expressing the Serotonin 5HT1A receptor are labeled with Terbium for conducting receptor binding studies on the aforementioned receptor.

The Tag-lite® Serotonin 5HT1A Receptor Ligand Binding Assay is a homogeneous alternative to radio ligand binding assays for HTS and compound profiling.

It is suitable for both saturation binding assays (Kd) and competitive binding assays (Ki). At equilibrium, the fraction of labeled ligand bound to the receptor is proportional to the FRET signal recorded. From this resulting signal, binding affinities can be calculated.

MATERIALS & EQUIPMENT

MATERIALS PROVIDED:

- Tag-lite Serotonin 5HT1A labeled Cells, ready-to-use (transformed & labeled), 200 tests*
(Part# C1TT15HT1A)

*Sufficient for 200 tests using a 96 or 384-well small volume white plate (20 µL). Purchase additional labeled cells for larger runs.

Notes:

1. Differences in Kd values may be observed between batches of labeled cells. Variability between Kd values reported in this package insert and values calculated during your experiment may also occur.
2. To ensure optimal reproducibility and consistency, single lot-bulk batches are available as a custom service. Our technical support team can help you set up this assay.

FOR KD AND KI DETERMINATION, PURCHASE SEPARATELY:

- Serotonin 5HT1A Receptor red antagonist Fluorescent Ligand (Revvity Part# L0029RED)
- Tag-lite Buffer (5X concentrate), 100 mL (Revvity Part# LABMED)
- Unlabeled ligand to measure non-specific signal: WAY 100,135 - (recommended)
- Microplates - For HTRF microplate recommendations, please visit www.revvity.com
- HTRF®-Certified Reader - For a list of HTRF-compatible readers and setup recommendations, please visit www.revvity.com

Use of an inappropriate set-up may seriously impair results. Check that you are using the set-up for Tb donor and red ligand. HTRF-approved readers using a monochromator for detection are not compatible with Tag-lite binding assays.

STORAGE AND HANDLING

Cells must be stored at -80°C or in liquid nitrogen until thawing. For storage > 1 month, store the frozen cells in liquid nitrogen.

Keep the cells frozen until all the other reagents are ready.

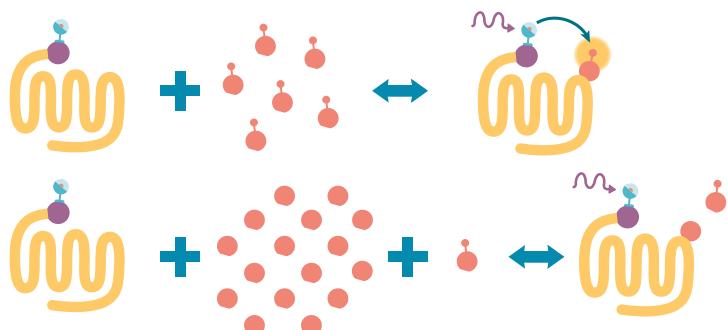
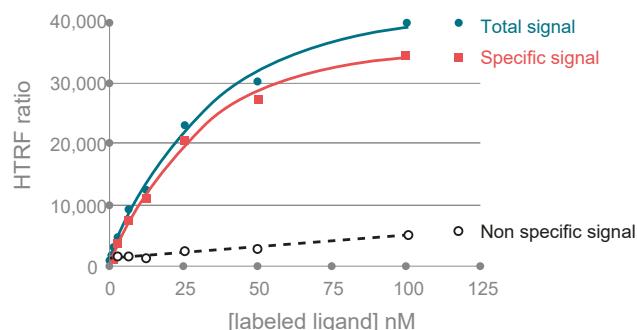
SATURATION BINDING (KD DETERMINATION)

A saturation binding assay measures total and non-specific binding of increasing concentrations of ligand under conditions of equilibrium.

To perform the assay, the fluorescent ligand is titrated into a solution containing a fixed amount of labeled cells and incubated to equilibrium. The HTRF ratio obtained from this titration is the total binding.

A negative control using unlabeled ligand is included to account for the non-specific binding of the labeled ligand to the receptor, non-receptor molecules, and the microplate. The fluorescent ligand is titrated into a solution containing a fixed amount of labeled cells and a 100-fold molar excess of unlabeled ligand. The HTRF ratio obtained from this titration is the non-specific binding.

The specific binding is calculated by subtracting the non-specific binding from the total binding at each fluorescent ligand concentration.



REAGENT PREPARATION

Step 1: prepare working Tag-lite buffer (1X TLB).

1. Determine the amount of 1 X TLB needed for the assay.
2. Thaw the 100 mL vial of Tag-lite buffer 5X (5X TLB).
3. Dilute 5-fold the 5X TLB in distilled water to prepare 1X TLB.
4. Mix gently.

Step 2: prepare fluorescent ligand.

The concentration of fluorescent ligand provided (Serotonin 5HT1A receptor red antagonist) is indicated on the vial label.

1. Centrifuge the vial
2. Dilute labeled ligand stock solution using 1X TLB to obtain the highest concentration **F1 = 1200 nM** for the saturation binding curve.

Use the following formula $C1V1 = C2V2$ to calculate the final volume needed to produce the **1200nM** solution

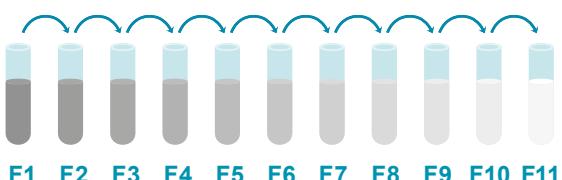
$V1 = (C2 \times V2) / C1$, where $V1$ is the final volume needed, $C1$ is the labeled ligand stock concentration, $C2$ is the labeled ligand desired concentration of 400nM, and $V2$ is the volume of stock labeled ligand.

Example for a ligand concentration $C_1 = 10\,000\text{ nM}$. Take 24 μL (V_1) of fluorescent ligand stock solution and add 176 μL of 1X TLB in order to obtain 200 μL (V_2) of 1200 nM (C_2) solution

3. Starting with the F1 solution (1200 nM), prepare 1/2 serial dilutions in 1X TLB.
4. Add 100 μL of F1 to 100 μL of 1X TLB, mix gently and repeat the 1/2 serial dilutions to prepare 600-1.2 nM solutions.

| RECOMMENDED DILUTION PROCEDURE FOR FLUORESCENT LIGAND | FLUORESCENT LIGAND CONCENTRATION (nM) | |
|---|--|--------------------------------|
| | INITIAL CONCENTRATIONS (WORKING SOLUTIONS) | FINAL CONCENTRATIONS (IN WELL) |
| F1 | Made from stock solution | 1200 |
| F2 | 100 μL F1 + 100 μL 1X TLB | 600 |
| F3 | 100 μL F2 + 100 μL 1X TLB | 300 |
| F4 | 100 μL F3 + 100 μL 1X TLB | 150 |
| F5 | 100 μL F4 + 100 μL 1X TLB | 75 |
| F6 | 100 μL F5 + 100 μL 1X TLB | 37.5 |
| F7 | 100 μL F6 + 100 μL 1X TLB | 18.8 |
| F8 | 100 μL F7 + 100 μL 1X TLB | 9.4 |
| F9 | 100 μL F8 + 100 μL 1X TLB | 4.7 |
| F10 | 100 μL F9 + 100 μL 1X TLB | 2.3 |
| F11 | 100 μL F10 + 100 μL 1X TLB | 1.2 |

Add 100 μL of TLB 1X from F2 to F11



F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11

Step 3: prepare unlabeled ligand

Prepare a working solution of unlabeled ligand WAY 100,135 in 1X TLB at 100-fold the concentration of F1 solution = 120 μ M. Please refer to literature accompanying the unlabeled ligand for stock concentration provided.

Step 4: prepare cells

1. Prepare a conical vial containing 5 mL of cold 1X TLB.
2. Thaw labeled frozen cells (1 vial) in a 37°C water bath - **manual shaking** - until all the ice is thawed (1-2 min).
3. Quickly transfer the cells by pipetting into the conical vial containing 1X TLB.
4. Centrifuge 5 min at 300 G. **The pellet may not be visible.**
5. Gently remove supernatant by aspiration. **Do not pour out the supernatant.**
6. Resuspend the pellet in 1 mL of 1X TLB. Mix gently by pipetting up and down several times.
7. Add 1.7 mL of 1X TLB. Mix gently by pipetting up and down several times.

Keep the cells at room temperature.

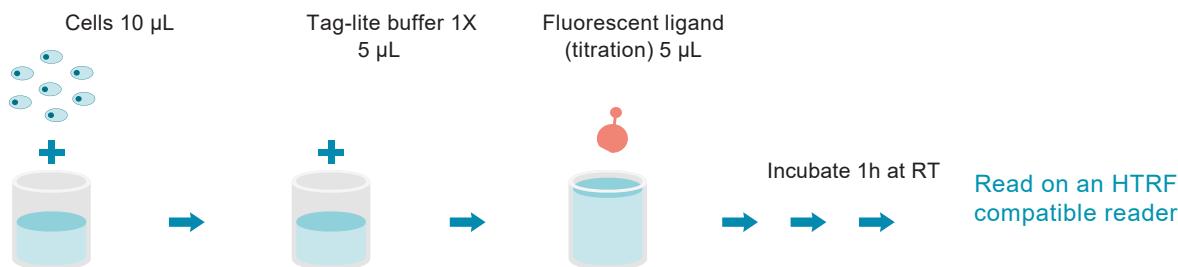
| Step 1 | Step 2 | Step 3 | Step 4 | Step 5 |
|---|--|---|---|---|
| Prepare a conical vial (A) containing 5 mL of cold 1X TLB | Thaw labeled frozen cells (1 vial) at 37°C (water bath, manual shaking) until all the ice is thawed (1-2 min) and transfer them quickly by pipetting into the vial prepared in Step 1. | Centrifuge 5 min at 300 G. Be careful the pellet may not be visible. | Gently remove supernatant by aspiration do not pour out supernatant. | Resuspend the pellet in 1 mL of 1X TLB. Mix gently by pipetting up and down several times. Add 1.7 mL of 1X TLB. Mix gently by pipetting up and down several times. Keep the cells at R.T. |

SATURATION BINDING ASSAY MANUAL

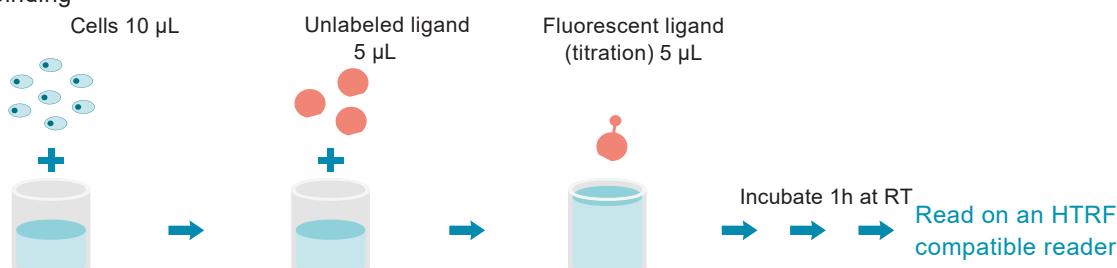
Run all assay points in triplicate. An example of plate map is indicated on page 4.

1. Dispense 10 μ L labeled cells into each well for both total and nonspecific binding.
2. Dispense 5 μ L 1X TLB into total binding wells.
3. Dispense 5 μ L unlabeled ligand (120 μ M) into nonspecific binding wells.
4. Dispense 5 μ L labeled ligand dilutions into each appropriate well.
5. Incubate 1h at room temperature.
6. Read on an HTRF-compatible reader - **HTRF-approved readers using a monochromator for detection are not compatible with Tag-lite binding assays.**

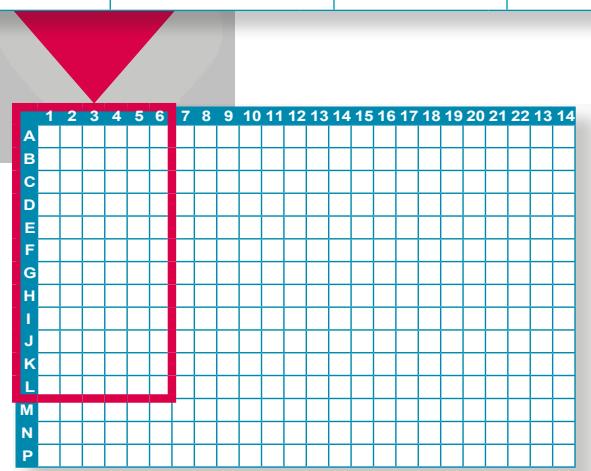
Total binding



Non specific binding

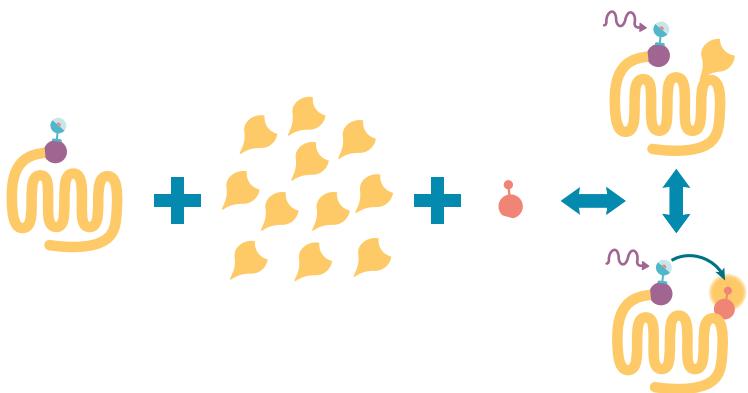
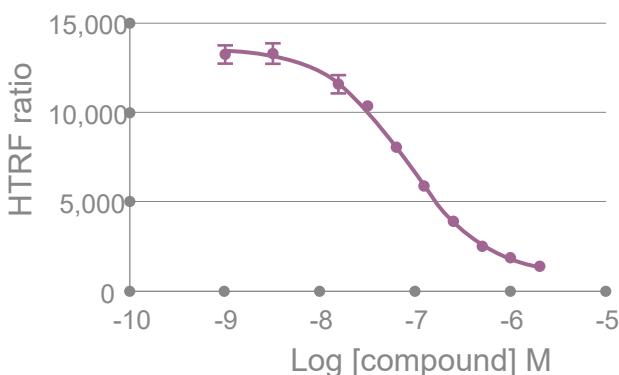


| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|----------------|----------------|--|----------------|----------------|
| A | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [1200 nM] | Repeat Well A1 | Repeat Well A1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [1200 nM] | Repeat Well A4 | Repeat Well A4 |
| B | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [600 nM] | Repeat Well B1 | Repeat Well B1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [600 nM] | Repeat Well B4 | Repeat Well B4 |
| C | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [300 nM] | Repeat Well C1 | Repeat Well C1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [300 nM] | Repeat Well C4 | Repeat Well C4 |
| D | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [150 nM] | Repeat Well D1 | Repeat Well D1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [150 nM] | Repeat Well D4 | Repeat Well D4 |
| E | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [75 nM] | Repeat Well E1 | Repeat Well E1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [75 nM] | Repeat Well E4 | Repeat Well E4 |
| F | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [37.5 nM] | Repeat Well F1 | Repeat Well F1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [37.5 nM] | Repeat Well F4 | Repeat Well F4 |
| G | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [18.8 nM] | Repeat Well G1 | Repeat Well G1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [18.8 nM] | Repeat Well G4 | Repeat Well G4 |
| H | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [9.4 nM] | Repeat Well H1 | Repeat Well H1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [9.4 nM] | Repeat Well H4 | Repeat Well H4 |
| I | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [4.7 nM] | Repeat Well I1 | Repeat Well I1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [4.7 nM] | Repeat Well I4 | Repeat Well I4 |
| J | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [2.3 nM] | Repeat Well J1 | Repeat Well J1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [2.3 nM] | Repeat Well J4 | Repeat Well J4 |
| K | 10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [1.2 nM] | Repeat Well K1 | Repeat Well K1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [1.2 nM] | Repeat Well K4 | Repeat Well K4 |
| L | 10 µL labeled cells 5 µL TLB 1X 5 µL TLB 1X (10 µL total TLB 1X) | Repeat Well L1 | Repeat Well L1 | 10 µL labeled cells 5 µL unlabeled ligand 5 µL TLB 1X | Repeat Well L4 | Repeat Well L4 |



COMPETITION BINDING (KI DETERMINATION)

Competitive binding assay is performed to measure the dissociation constant, K_i . To perform the assay, the compound is titrated into a solution containing a fixed concentration of fluorescent ligand and a fixed amount of cells.



REAGENT PREPARATION

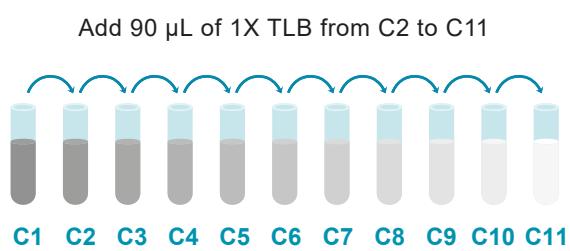
Step 1: prepare working tag-lite buffer (1X TLB).

1. Determine the amount of 1X TLB needed for the assay. (Approximately 10 mL is required to assay one compound + 1 mL for each additional compound.)
2. Thaw the 100 mL vial of Tag-lite buffer 5X (5X TLB).
3. Dilute 5-fold the 5X TLB in distilled water to prepare 1X TLB. (E.g. 10 mL of 5X TLB + 40 mL distilled water.)
4. Mix gently.

Step 2: prepare compounds

1. Dilute compounds with 1X TLB to an initial concentration of 4.E-04 M (C1).
2. Starting with the C1 solution (4.E-04 M), prepare 1/10 serial dilutions in 1X TLB.
3. Add 10 μ L C1 to 90 μ L of 1X TLB, mix gently and repeat the 1/10 serial dilutions to prepare C2, C3, C4, C5, C6, C7, C8, C9, C10, C11 solutions.

| RECOMMENDED DILUTION PROCEDURE FOR COMPOUNDS | COMPOUND CONCENTRATIONS (M) | |
|--|--|--------------------------------|
| | INITIAL CONCENTRATIONS (WORKING SOLUTIONS) | FINAL CONCENTRATIONS (IN WELL) |
| C1 | Made from stock compounds | 4.E-04 |
| C2 | 10 μ L C1 + 90 μ L 1X TLB | 4.E-05 |
| C3 | 10 μ L C2 + 90 μ L 1X TLB | 4.E-06 |
| C4 | 10 μ L C3 + 90 μ L 1X TLB | 4.E-07 |
| C5 | 10 μ L C4 + 90 μ L 1X TLB | 4.E-08 |
| C6 | 10 μ L C5 + 90 μ L 1X TLB | 4.E-09 |
| C7 | 10 μ L C6 + 90 μ L 1X TLB | 4.E-10 |
| C8 | 10 μ L C7 + 90 μ L 1X TLB | 4.E-11 |
| C9 | 10 μ L C8 + 90 μ L 1X TLB | 4.E-12 |
| C10 | 10 μ L C9 + 90 μ L 1X TLB | 4.E-13 |
| C11 | 10 μ L C10 + 90 μ L 1X TLB | 4.E-14 |



Step 3: prepare fluorescent ligand

For the competition dose-response of compounds, the optimal fluorescent ligand concentration is the one that allows 50% (K_d) to 80% of receptor binding.

The concentration of fluorescent ligand Serotonin 5HT1A receptor red antagonist is indicated on the vial label⁸⁰.

Centrifuge the vial then dilute the fluorescent ligand Serotonin 5HT1A receptor red antagonist with 1X TLB

step 4: prepare cells

1. Prepare a conical vial containing 5 mL of cold 1X TLB.
2. Thaw labeled frozen cells (1 vial) in a 37°C water bath (manual shaking) until all the ice is thawed (1-2 min).
3. Quickly transfer them by pipetting into the conical vial containing 1X TLB.
4. Centrifuge 5 min at 300 G. (The pellet may not be visible.)
5. Gently remove supernatant by aspiration. (Do not pour out the supernatant.)
6. Resuspend the pellet in 1 mL of 1X TLB. Mix gently by pipetting up and down several times.
7. Add 1.7 mL of 1X TLB. Mix gently by pipetting up and down several times.
8. Keep the cells at room temperature

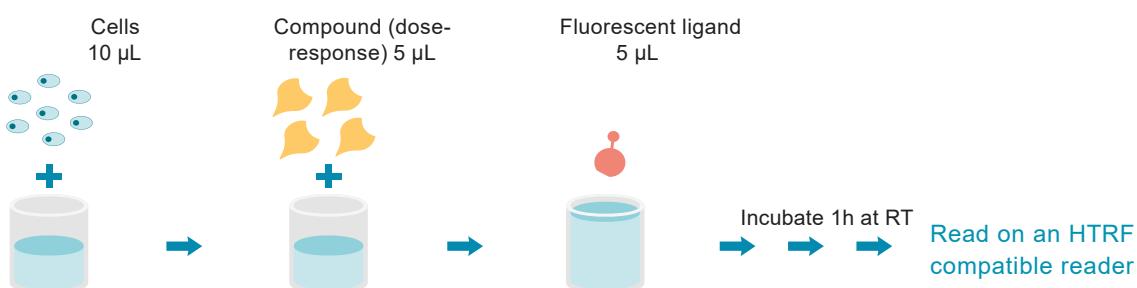
| Step 1 | Step 2 | Step 3 | Step 4 | Step 5 |
|---|---|--|---|--|
| Prepare a conical vial (A) containing 5 mL of cold 1X TLB | Thaw labeled frozen cells (1 vial) at 37°C (water bath, manual shaking) until all the ice is thawed (1-2 min) and transfer them quickly by pipeting into the vial prepared in Step 1. | Centrifuge 5 min at 300 G.  Be careful the pellet may not be visible. | Gently remove supernatant by aspiration do not pour out supernatant. | Resuspend the pellet in 1 mL of 1X TLB. Mix gently by pipetting up and down several times. Add 1.7 mL of 1X TLB. Mix gently by pipetting up and down several times. Keep the cells at R.T.  |

COMPETITIVE BINDING ASSAY MANUAL

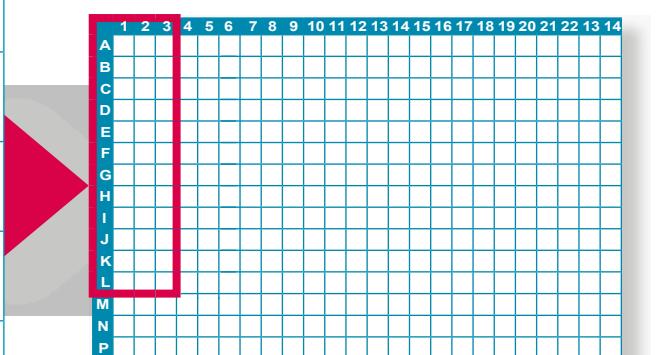
Run all assay points in triplicate. An example of plate map is indicated on page 7.

Up to ten (10) compounds can be tested in one 384-well plate for a total of 36 wells per compound.

1. Dispense 10 μ L labeled cells into each well.
2. Dispense 5 μ L 1X TLB or 5 μ L compound dilutions into each appropriate well as shown.
3. Repeat for each compound tested.
4. Dispense 5 μ L labeled ligand into each well.
5. Incubate 1h at room temperature.
6. Read on an HTRF-compatible reader - **HTRF-approved readers using a monochromator for detection are not compatible with Tag-lite binding assays.**



| | 1 | 2 | 3 |
|---|---|----------------|----------------|
| A | Compound 1 10 μ L labeled cells 5 μ L compound [C1] 5 μ L labeled ligand | Repeat Well A1 | Repeat Well A1 |
| B | 10 μ L labeled cells 5 μ L compound [C2] 5 μ L labeled ligand | Repeat Well B1 | Repeat Well B1 |
| C | 10 μ L labeled cells 5 μ L compound [C3] 5 μ L labeled ligand | Repeat Well C1 | Repeat Well C1 |
| D | 10 μ L labeled cells 5 μ L compound [C4] 5 μ L labeled ligand | Repeat Well D1 | Repeat Well D1 |
| E | 10 μ L labeled cells 5 μ L compound [C5] 5 μ L labeled ligand | Repeat Well E1 | Repeat Well E1 |
| F | 10 μ L labeled cells 5 μ L compound [C6] 5 μ L labeled ligand | Repeat Well F1 | Repeat Well F1 |
| G | 10 μ L labeled cells 5 μ L compound [C7] 5 μ L labeled ligand | Repeat Well G1 | Repeat Well G1 |
| H | 10 μ L labeled cells 5 μ L compound [C8] 5 μ L labeled ligand | Repeat Well H1 | Repeat Well H1 |
| I | 10 μ L labeled cells 5 μ L compound [C9] 5 μ L labeled ligand | Repeat Well I1 | Repeat Well I1 |
| J | 10 μ L labeled cells 5 μ L compound [C10] 5 μ L labeled ligand | Repeat Well J1 | Repeat Well J1 |
| K | 10 μ L labeled cells 5 μ L compound [C11] 5 μ L labeled ligand | Repeat Well K1 | Repeat Well K1 |
| L | 10 μ L labeled cells 5 μ L TLB 1X 5 μ L labeled ligand | Repeat Well L1 | Repeat Well L1 |



DATA REDUCTION

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

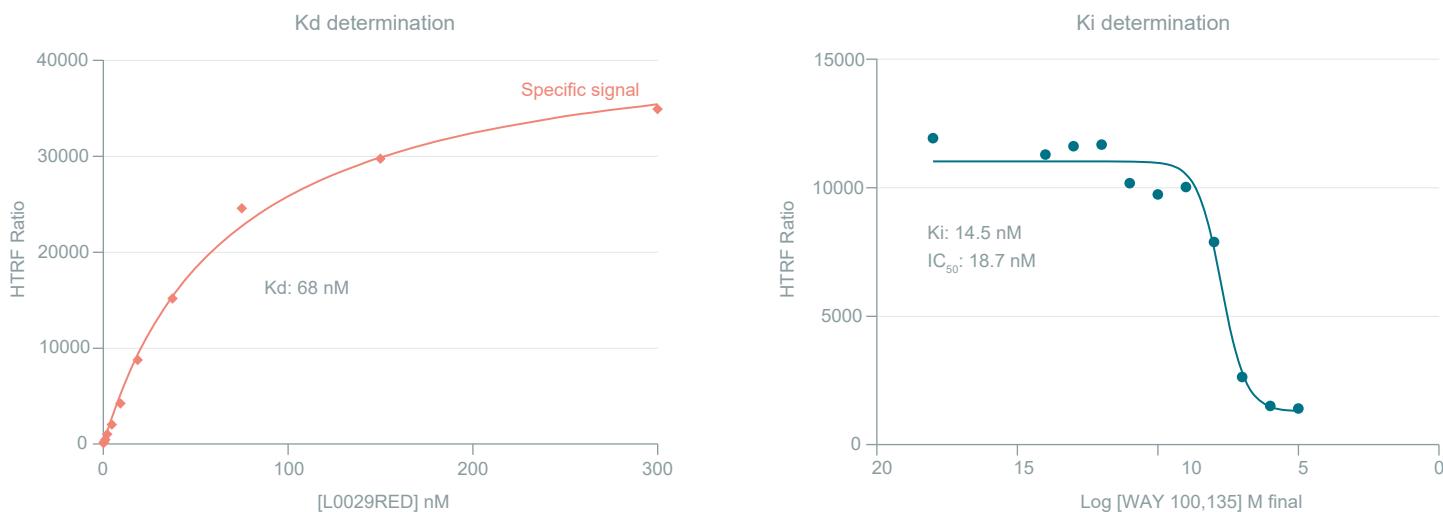
$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Plot the HTRF ratio versus the [fluorescent ligand] or [compound] concentrations.

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

RESULTS

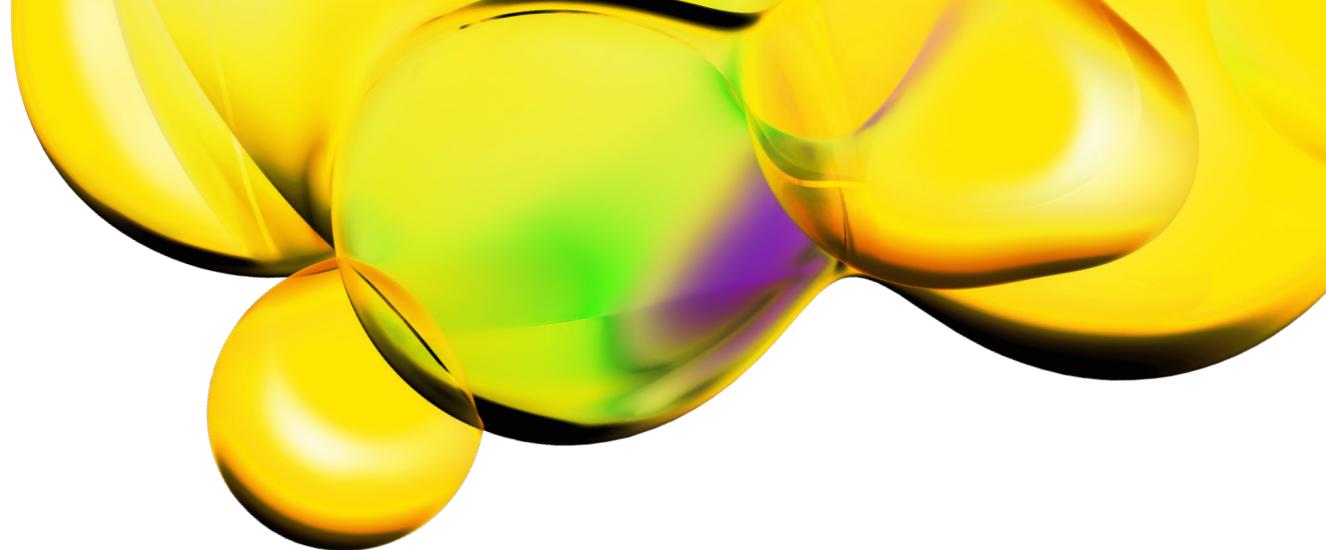
Example of data obtained for Serotonin 5HT1A receptor with WAY 100,135 as reference ligand and readings taken on a PHERAstarFS with flash laser. Results may vary from one HTRF® compatible reader to another.



Notes:

1. Differences in Kd values may be observed between batches of labeled cells. Variability between Kd values reported in this package insert and values calculated during your experiment may also occur.
2. To ensure optimal reproducibility and consistency, single lot-bulk batches are available as a custom service. Our technical support team can help you set up this assay.

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