MANUAL

DOPAMINE D2 LABELED CELLS

Dopamine D2 labeled cells for: 200 tests

Part#: C1TT1D2

Revision : #02 of September 2023

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 Dopamine D2 cells transiently expressing the Dopamine D2 receptor are labeled with Terbium for conducting receptor binding studies on the aforementioned receptor.

The Tag-lite® Dopamine D2 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 Dopamine D2 labeled Cells, ready-to-use (transformed & labeled), 200 tests* (Part# C1TT1D2)

*Sufficient for 200 tests 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:

- Dopamine D2 Receptor red antagonist Fluorescent Ligand (Revvity Part# L0002RED)
- Tag-lite Buffer (5X concentrate), 100 mL (Revvity Part# LABMED)
- Unlabeled ligand to measure non-specific signal: Bromocriptine - (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.

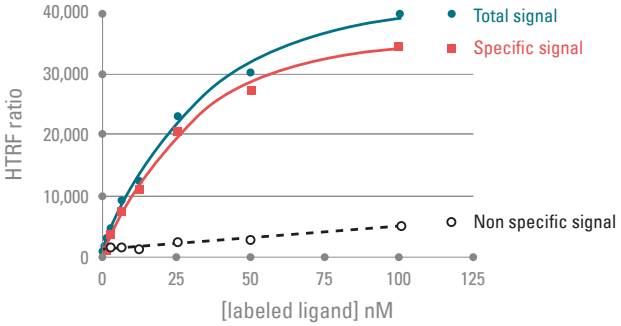
2 SATURATION BINDING (KD DETERMINATION)

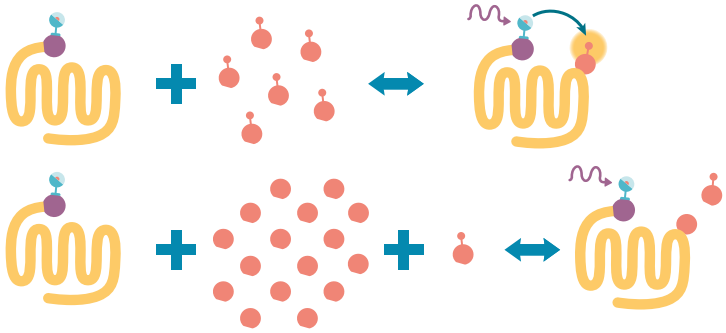
A saturation binding assay measures total and non-specific binding of increasing concentrations of ligand under conditions of equilibrium.

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.

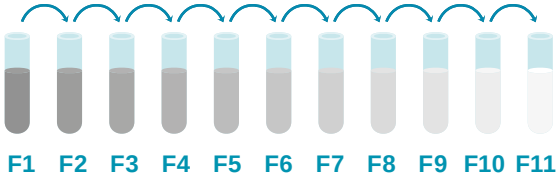
Step 2: prepare fluorescent ligand.

The concentration of fluorescent ligand provided (Dopamine D2 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 = 400 nM for the saturation binding curve.
   Use the following formula C1V1 = C2V2 to calculate the final volume needed to produce the 400nM solution
   V1 = (C2 x 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 nM. Take 8 µL (V_1) of fluorescent ligand stock solution and add 192 µL of 1X TLB in order to obtain 200 µL (V_2) of 400 nM (C_2) solution
3. Starting with the F1 solution (400 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 200-0.4 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	400	100
F2	100 µL F1 + 100 µL 1X TLB	200	50
F3	100 µL F2 + 100 µL 1X TLB	100	25
F4	100 µL F3 + 100 µL 1X TLB	50	12.5
F5	100 µL F4 + 100 µL 1X TLB	25	6.3
F6	100 µL F5 + 100 µL 1X TLB	12.5	3.1
F7	100 µL F6 + 100 µL 1X TLB	6.3	1.6
F8	100 µL F7 + 100 µL 1X TLB	3.1	0.8
F9	100 µL F8 + 100 µL 1X TLB	1.6	0.4
F10	100 µL F9 + 100 µL 1X TLB	0.8	0.2
F11	100 µL F10 + 100 µL 1X TLB	0.4	0.1

Add 100 µL of TLB 1X from F2 to F11



Step 3: prepare unlabeled ligand

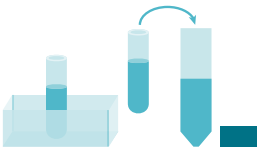
Prepare a working solution of unlabeled ligand Bromocriptine in 1X TLB at 100-fold the concentration of F1 solution = 40 µM.

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.

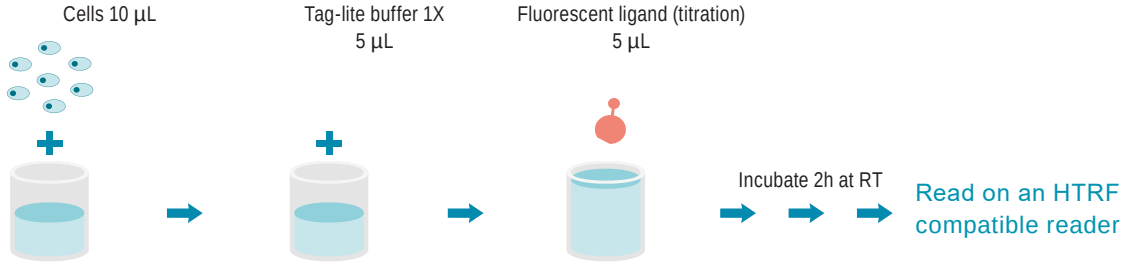
Step 1	Step 2	Step 3	Step 4	Step 5
Prepare a conical vial (A) containing 5 mL of cold 1X TLB 	Thaw labeled frozen cells (1vial) at 37°C (water bath, manual shaking) until all the ice is thawed (1-2 min) and transfer them quickly by pipeting into the vial prepared in Step 1. 	Centrifuge 5 min at 300 G.  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. 

SATURATION BINDING ASSAY MANUAL

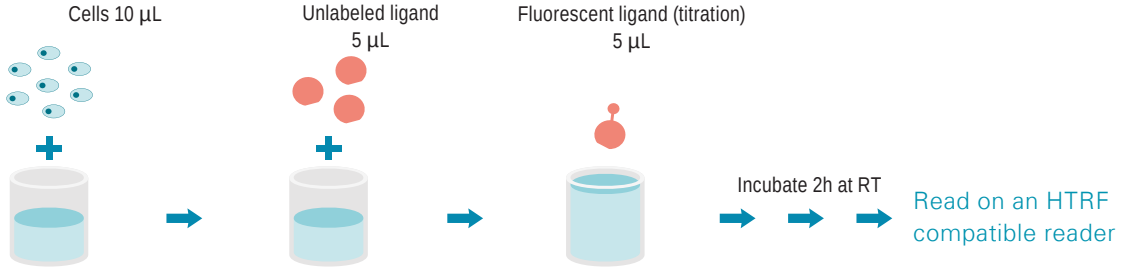
Run all assay points in triplicate. An example of plate map is indicated on page 4.

1. Dispense 10 µL labeled cells into each well for both total and nonspecific binding.
2. Dispense 5 µL 1X TLB into total binding wells.
3. Dispense 5 µL unlabeled ligand (40 µM) into nonspecific binding wells.
4. Dispense 5 µL labeled ligand dilutions into each appropriate well.
5. Incubate 2h 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.

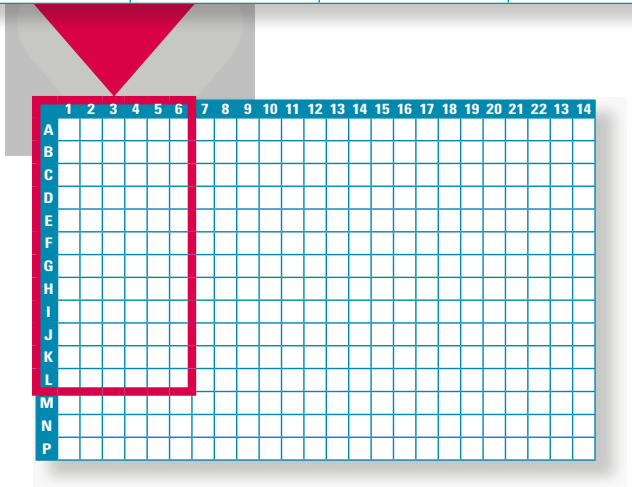
Total binding



Non specific binding

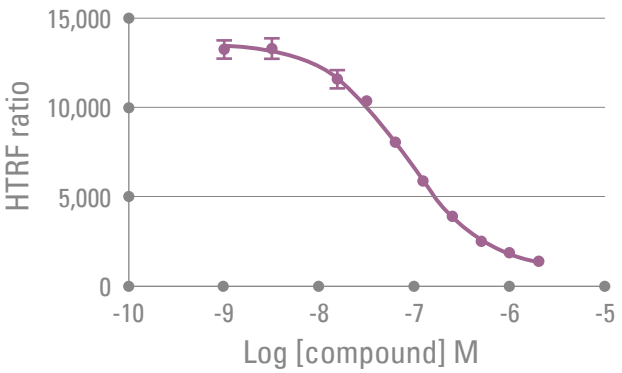


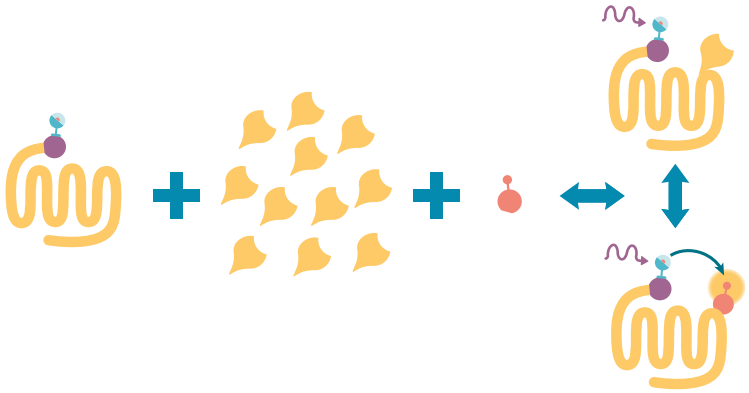
	1	2	3	4	5	6
A	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [400 nM]	Repeat Well A1	Repeat Well A1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [400 nM]	Repeat Well A4	Repeat Well A4
B	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [200 nM]	Repeat Well B1	Repeat Well B1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [200 nM]	Repeat Well B4	Repeat Well B4
C	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [100 nM]	Repeat Well C1	Repeat Well C1	10 µL labeled cells 5 µLunlabeled ligand 5 µL labeled ligand [100 nM]	Repeat Well C4	Repeat Well C4
D	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [50 nM]	Repeat Well D1	Repeat Well D1	10 µL labeled cells 5 µLunlabeled ligand 5 µL labeled ligand [50 nM]	Repeat Well D4	Repeat Well D4
E	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [25 nM]	Repeat Well E1	Repeat Well E1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [25 nM]	Repeat Well E4	Repeat Well E4
F	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [12.5 nM]	Repeat Well F1	Repeat Well F1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [12.5 nM]	Repeat Well F4	Repeat Well F4
G	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [6.3 nM]	Repeast Well G1	Repeast Well G1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [6.3 nM]	Repeast Well G4	Repeast Well G4
H	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [3.1 nM]	Repeat Well H1	Repeat Well H1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [3.1 nM]	Repeat Well H4	Repeat Well H4
I	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [1.6 nM]	Repeat Well I1	Repeat Well I1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [1.6 nM]	Repeat Well I4	Repeat Well I4
J	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [0.8 nM]	Repeat Well J1	Repeat Well J1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [0.8 nM]	Repeat Well J4	Repeat Well J4
K	10 µL labeled cells 5 µL TLB 1X 5 µL labeled ligand [0.4 nM]	Repeat Well K1	Repeat Well K1	10 µL labeled cells 5 µL unlabeled ligand 5 µL labeled ligand [0.4 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, Ki. 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).

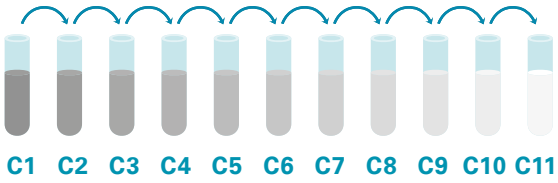
1. Determine the amount of 1X TLB needed for the assay. (Approximately 10 mL is required to assay one compound + 1 mL for each additional compound.)
2. Thaw the 100 mL vial of Tag-lite buffer 5X (5X TLB).
3. Dilute 5-fold the 5X TLB in distilled water to prepare 1X TLB. (E.g. 10 mL of 5X TLB + 40 mL 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 µL C1 to 90 µL 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	1.E-04
C2	10 µL C1 + 90 µL 1X TLB	4.E-05	1.E-05
C3	10 µL C2 + 90 µL 1X TLB	4.E-06	1.E-06
C4	10 µL C3 + 90 µL 1X TLB	4.E-07	1.E-07
C5	10 µL C4 + 90 µL 1X TLB	4.E-08	1.E-08
C6	10 µL C5 + 90 µL 1X TLB	4.E-09	1.E-09
C7	10 µL C6 + 90 µL 1X TLB	4.E-10	1.E-10
C8	10 µL C7 + 90 µL 1X TLB	4.E-11	1.E-11
C9	10 µL C8 + 90 µL 1X TLB	4.E-12	1.E-12
C10	10 µL C9 + 90 µL 1X TLB	4.E-13	1.E-13
C11	10 µL C10 + 90 µL 1X TLB	4.E-14	1.E-14

Add 90 µL of 1X TLB from C2 to C11



step 3: prepare fluorescent ligand

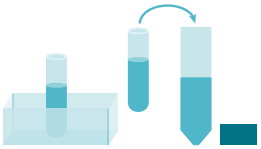
For the competition dose-response of compounds, the optimal fluorescent ligand concentration is the one that allows 50% (Kd) to 80% of receptor binding.

The concentration of fluorescent ligand Dopamine D2 receptor red antagonist is indicated on the vial label12.

Centrifuge the vial then dilute the fluorescent ligand Dopamine D2 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

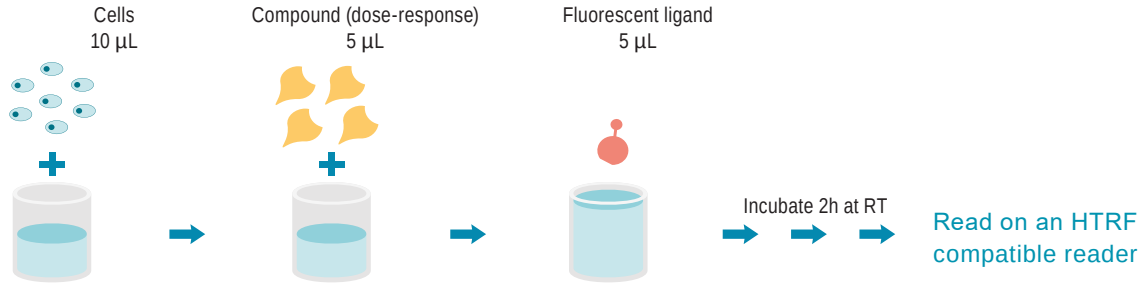
Step 1	Step 2	Step 3	Step 4	Step 5
Prepare a conical vial (A) containing 5 mL of cold 1X TLB 	Thaw labeled frozen cells (1vial) at 37°C (water bath, manual shaking) until all the ice is thawed (1-2 min) and transfer them quickly by pipeting into the vial prepared in Step 1. 	Centrifuge 5 min at 300 G.  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

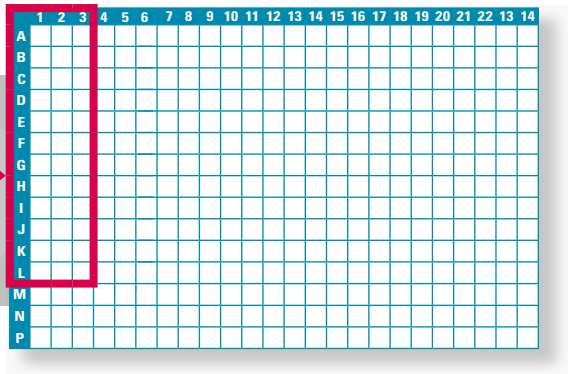
Run all assay points in triplicate. An example of plate map is indicated on page 7.

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

1. Dispense 10 µL labeled cells into each well.
2. Dispense 5 µL 1X TLB or 5 µL compound dilutions into each appropriate well as shown.
3. Repeat for each compound tested.
4. Dispense 5 µL labeled ligand into each well.
5. Incubate 2h 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 µL labeled cells 5 µL compound [C1] 5 µL labeled ligand	Repeat Well A1	Repeat Well A1
B	10 µL labeled cells 5 µL compound [C2] 5 µL labeled ligand	Repeat Well B1	Repeat Well B1
C	10 µL labeled cells 5 µL compound [C3] 5 µL labeled ligand	Repeat Well C1	Repeat Well C1
D	10 µL labeled cells 5 µL compound [C4] 5 µL labeled ligand	Repeat Well D1	Repeat Well D1
E	10 µL labeled cells 5 µL compound [C5] 5 µL labeled ligand	Repeat Well E1	Repeat Well E1
F	10 µL labeled cells 5 µL compound [C6] 5 µL labeled ligand	Repeat Well F1	Repeat Well F1
G	10 µL labeled cells 5 µL compound [C7] 5 µL labeled ligand	Repeat Well G1	Repeat Well G1
H	10 µL labeled cells 5 µL compound [C8] 5 µL labeled ligand	Repeat Well H1	Repeat Well H1
I	10 µL labeled cells 5 µL compound [C9] 5 µL labeled ligand	Repeat Well I1	Repeat Well I1
J	10 µL labeled cells 5 µL compound [C10] 5 µL labeled ligand	Repeat Well J1	Repeat Well J1
K	10 µL labeled cells 5 µL compound [C11] 5 µL labeled ligand	Repeat Well K1	Repeat Well K1
L	10 µL labeled cells 5 µL TLB 1X 5 µL 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.

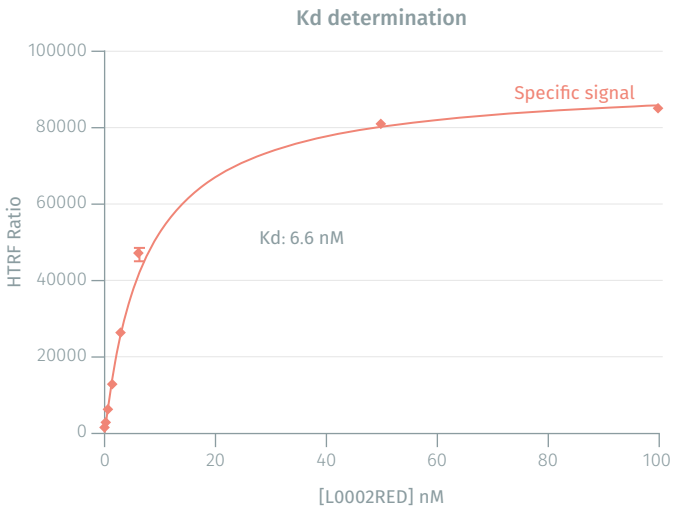
$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

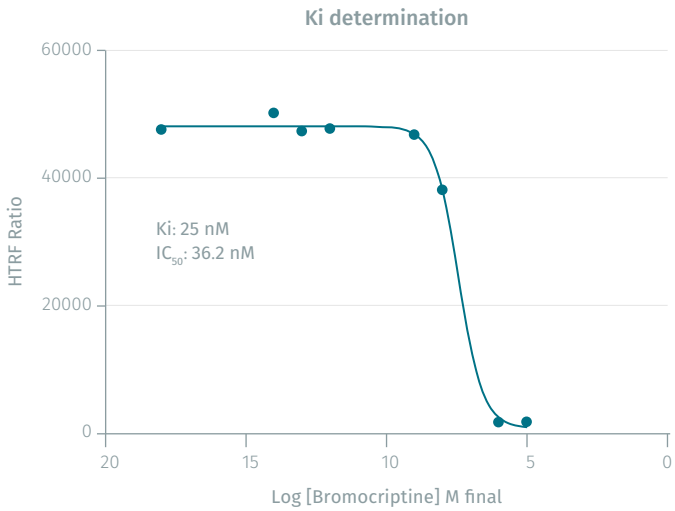
2. Plot the HTRF ratio versus the [fluorescent ligand] or [compound] concentrations.

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

RESULTS

Example of data obtained for Dopamine D2 receptor with Bromocriptine as reference ligand and readings taken on a PHERAstarFS with flash lamp. Results may vary from one HTRF® compatible reader to another.

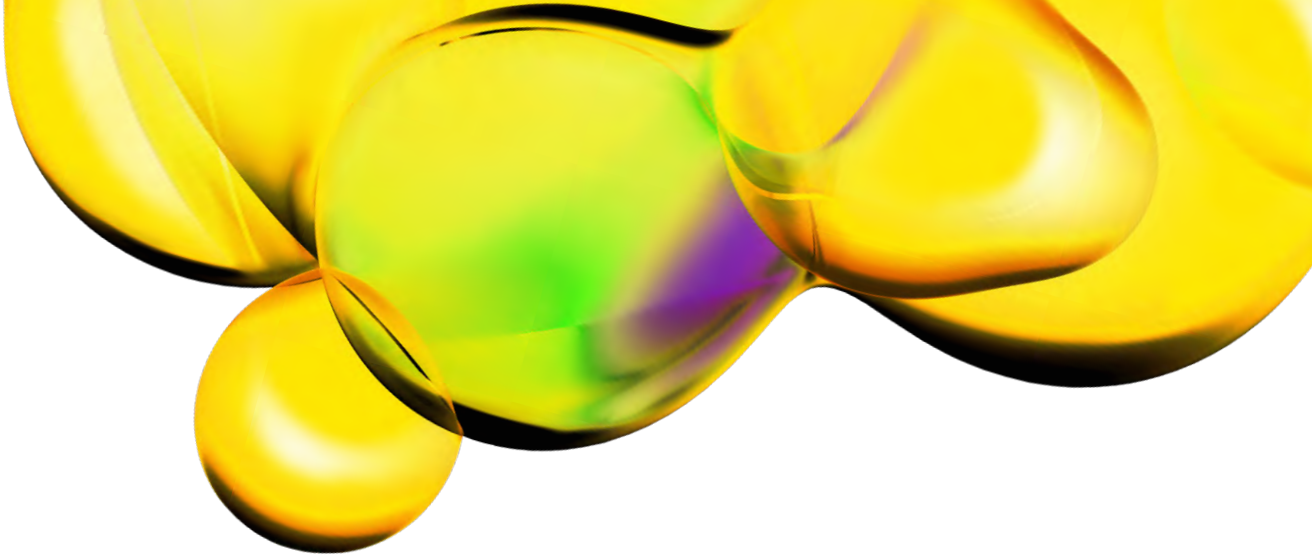




Notes:

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.

This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage. The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact. The product is genetically modified and must be used according to biosafety level S1.The Tag-lite® Dopamine D2 cells are derived from a HEK 293 background genetically modified to transiently express the Human Dopamine D2. As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 01/18/EC on the contained use of genetically modified organisms.



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

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