MANUAL

HTRF IP-One Gq Detection Kit

IP-ONE - Gq KIT:

Part #: 62IPAPEB (1,000 tests), 62IPAPEC (20,000 tests), 62IPAPEJ (100,000 tests)

Version: #10 of September 2024

Storage temperature: 2-8°C

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

ASSAY PRINCIPLE

Revvity's IP-One Gq kit is a competitive immunoassay intended to measure myo-Inositol 1 phosphate (IP1) accumulation in cells. It enables the direct pharmacological characterization of compounds acting on G_q-coupled receptors in either adherent or suspension cells.

The principle is based on HTRF® technology. Native IP1 produced by cells or unlabeled IP1 (standard curve) compete with d2-labeled IP1 (acceptor) for binding to anti-IP1-Cryptate (donor). The specific signal (i.e. energy transfer) is inversely proportional to the concentration of IP1 in the standard or sample (Fig. 1).

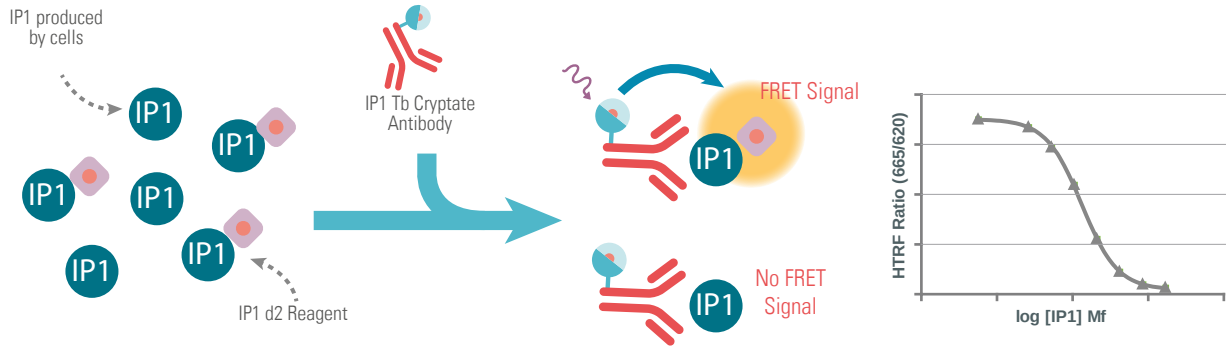


Figure 1: Principle of HTRF IP1 competitive binding assay.

As for all other HTRF assays, the calculation of the fluorescence ratio (665 nm/620 nm) eliminates possible medium interferences and means the assay is unaffected by the usual experimental conditions (e.g. culture medium, serum, biotin, colored compounds, etc.).

MATERIALS & EQUIPMENT

MATERIALS PROVIDED:

NUMBER OF TESTS (CAT. #)	ABBREVIATION	62IPAPEB (1,000 tests)	62IPAPEC (20,000 tests)	62IPAPEJ (100,000 tests)
IP1 Tb Cryptate Antibody (donor, lyophilized)	Ab-Cryp	1 vial	1 vial	5 vials
IP1 d2 Reagent (acceptor, lyophilized)	IP1-d2	1 vial	1 vial	5 vials
IP-One - Gq standard (lyophilized)	Std	1 vial	1 vial	1 vial
Lysis & Detection Buffer 5*	Lysis Buffer 5	1 vial (13 mL)	-	-
Lysis & Detection Buffer 6*	Lysis Buffer 6	-	1 vial (200 mL)	5 vials (200 mL each)
Stimulation Buffer 2 (5X)**	StimB	1 vial (8 mL)	1 vial (100 mL)	5 vials (100 mL each)

* The Lysis & Detection Buffer is used to prepare working solutions of acceptor and donor reagents.

** It is used to dilute the IP-One - Gq standards, test compounds and cells.

PURCHASE SEPARATELY:

- Small volume plates: Visit www.revvity.com for more information*
- HTRF®-Certified Reader**

* Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.
** For a list of HTRF-compatible readers and set-up recommendations, visit www.revvity.com

STORAGE AND STABILITY

Store the kit at 2-8°C until the expiration date indicated on the package.

Once reconstituted, stock solutions are stable at 2-8°C for 6 days. Stored at ≤-16°C, they are stable for 3 months. Stock solutions may be frozen and thawed only once. Freeze in aliquots to avoid multiple freeze/thaw cycles.

Working solutions of detection reagents (1X) and standards (for standard dose response curve) can be stored at 2-8°C for up to 6 days. Stored at -20°C, they are stable for 3 months

Note: IP1 Tb Cryptate Antibody working solutions (prepared with frozen stock solutions) should be filtered before use to improve assay reproducibility.

REAGENT PREPARATION

BEFORE YOU BEGIN:

1. *It is very important to prepare reagents in the buffer specified. Use of an incorrect buffer may affect reagent stability and assay results.*
2. *Allow the lyophilized reagents to warm up to room temperature for at least 30 mins before reconstitution.*
3. *IP1 standards (for standard curve) must be prepared in stimulation buffer 2 diluted at 1X (Supplied StimB 5X).*
4. *Detection reagents concentrations have been set for optimal assay performances. Note that any dilution or improper use of d2 and cryptate Tb detection reagents will impair the assay quality.*
5. *Please note that this kit use a Terbium donor crypate, make sure to set up your reader for Terbium cryptate.*

Take care to prepare stock and working solutions according to the directions below for the kit size you have purchased.

TO PREPARE STOCK SOLUTIONS:

If the kit is not going to be used at once please consider aliquoting the stock solutions before freezing considering solutions can only be frozen/thawed once.

1,000 TESTS KIT	20,000/100,000 TESTS KITS
1. Reconstitute the IP1 Tb Cryptate Antibody (donor) with 0.5 mL distilled water. Mix gently. *This is a 6X stock solution which can be stored for 6 days at 2-8°C or frozen up to 3 months.*	1. Reconstitute the IP1 Tb Cryptate Antibody (donor) with 3mL distilled water. Mix gently. *This is a 20X stock solution which can be stored for 6 days at 2-8°C or frozen up to 3 months.*
2. Reconstitute the IP1 d2 Reagent (acceptor) with 0.5 mL distilled water. Mix gently. *This is a 6X stock solution which can be stored for 6 days at 2-8°C or frozen up to 3 months*	2. Reconstitute the IP1 d2 Reagent (acceptor) with 3 mL distilled water. Mix gently. *This is a 20X stock solution which can be stored for 6 days at 2-8°C or frozen up to 3 months.*
3. Reconstitute the IP-One - Gq standard using distilled water. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. *The Standard Concentrate is stable frozen for 3 months at -20°C.*	3. Reconstitute the IP-One - Gq standard using distilled water. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. *The Standard Concentrate is stable frozen for 3 months at -20°C.*

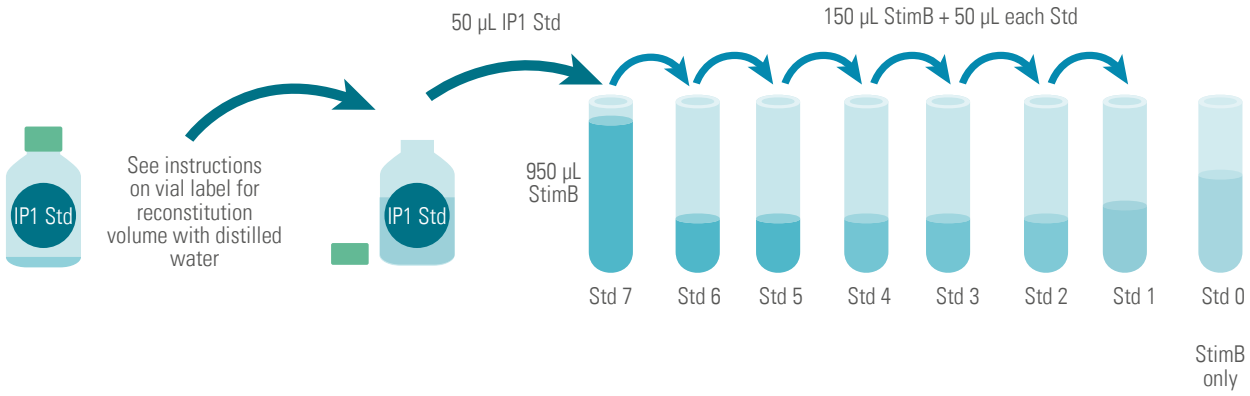
TO PREPARE WORKING DETECTION REAGENT SOLUTIONS:

1,000 TESTS KIT	20,000/100,000 TESTS KITS
1. Stimulation Buffer n°2: From the 5X stock solution: dilute 1 volume of StimB in 4 volumes of distilled water.	1. Stimulation Buffer n°2: From 5X stock solution: dilute 1 volume of StimB in 4 volumes of distilled water.
2. Detection reagents: From the 6X stock solutions (reconstituted reagents): For each detection reagent (IP1 Tb Cryptate Antibody and IP1 d2 Reagent), and in separate vials, dilute 1 volume of stock solutions in 5 volumes of Lysis & Detection Buffer 5 (e.g., 0.5 mL of reconstituted reagent + 2.5 mL of Lysis & Detection Buffer).	2. Detection reagents: From the 20X stock solutions (reconstituted reagents): For each detection reagent (IP1 Tb Cryptate Antibody and IP1 d2 Reagent), and in separate vials, dilute 1 volume of stock solutions in 19 volumes of Lysis & Detection Buffer 6 (e.g., 0.5 mL of reconstituted reagent + 9.5 mL of Lysis & Detection Buffer)

STANDARD CURVE

Run a standard dose response curve to determine the linear dynamic range of the assay. This will also verify that your assay is generating the expected S/B and IC50. In particular, the IC10 and IC90 will be useful in experiments to optimize the cell density of the cell lines you will stimulate with compounds.

TO PREPARE WORKING STANDARDS:



STANDARD	SERIAL DILUTIONS	IP1 WORKING SOLUTION nM	IP1 FINAL CONCENTRATION nM
Std7	50 µL IP1 Std reconstitued + 950 µL StimB*	11000	7700
Std6	50 µL Std7 + 150 µL StimB	2750	1925
Std5	50 µL Std6 + 150 µL StimB	688	481.6
Std4	50 µL Std5 + 150 µL StimB	172	120.4
Std3	50 µL Std4 + 150 µL StimB	43	30.1
Std2	50 µL Std3 + 150 µL StimB	11	7.7
Std1	50 µL Std2 + 150 µL StimB	2.7	1.9
Std0 (positive control)	150 µL StimB	0	0

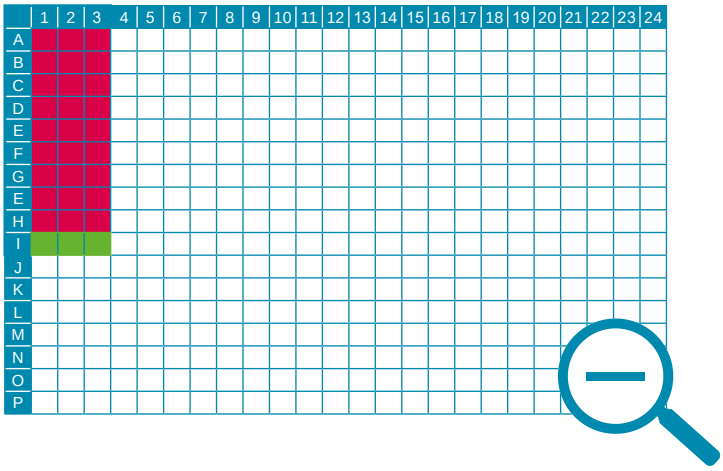
*StimB has to be first diluted with distilled water from 5X to a 1X solution (e.g. 1 Volume of StimB 5X + 4 volumes of distilled water).

STANDARD CURVE ASSAY MANUAL (384W LV PLATE)

	STANDARD (Std7-Std0)	NEGATIVE CONTROL
Step 1	Dispense 14 µL of each IP1 standard (Std7-Std0) into each standard well, in triplicate.	Dispense 14 µL StimB into negative control wells, in triplicate.
Step 2	Add 3 µL of IP1 d2 Reagent working solution to all wells containing standard.	Add 3 µL of Lysis & Detection Buffer to all wells containing negative control.
Step 3	Add 3 µL of IP1 Tb Cryptate Antibody working solution to all wells.	Add 3 µL of IP1 Tb Cryptate Antibody working solution to all wells.
Step 4	Seal the plate and incubate 1 hour at room temperature.	Seal the plate and incubate 1 hour at room temperature.
Step 5	Remove the plate seal and read on an HTRF compatible reader.	Remove the plate seal and read on an HTRF compatible reader.

Suggested plate map:

	1	2	3
A	14 µL Std 0 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well A1	Repeat Well A1
B	14 µL Std 1 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well B1	Repeat Well B1
C	14 µL Std 2 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well C1	Repeat Well C1
D	14 µL Std 3 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well D1	Repeat Well D1
E	14 µL Std 4 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well E1	Repeat Well E1
F	14 µL Std 5 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well F1	Repeat Well F1
G	14 µL Std 6 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well G1	Repeat Well G1
H	14 µL Std 7 3 µL IP1 d2 Reagent 3 µL IP1 Tb Cryptate Antibody	Repeat Well H1	Repeat Well H1
I	14 µL Stim. Buffer 3 µL Lysis & Detection Buffer 3 µL IP1 Tb Cryptate Antibody	Repeat Well I1	Repeat Well I1



Standard curve

Negative control (e.g. no IP1 d2 Reagent)

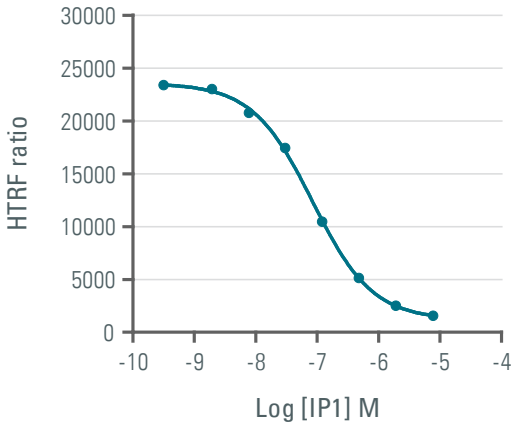
DATA REDUCTION & INTERPRETION

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 IP1 final concentrations.

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



Characteristics of the assay relative to the IC_{50} and the signal over background.

Obtained using the PHERAstar Plus reader (BMG LABTECH).

	IC_{50} (nM)	S/B
Incubation 1 hour at RT	100	20

CELL-BASED ASSAY

BEFORE RUNNING A CELL-BASED ASSAY:

1. *The kit is designed to work with the provided stimulation buffer, which we recommend using. If you wish to use your own stimulation buffer, please refer to our Guides to optimizing IP-One assays (available on Revvity's website for free) for additional recommendations and make sure your buffer meets the assay requirements.*
2. *Cell density must be also optimized to ensure that IP1 levels of unstimulated and stimulated cells fall within the linear dynamic range of the assay (IC10 to IC90). If results fall outside the assay's linear range, the data will be inaccurate.*
3. *Stimulation time needs to be optimized.*
4. *HTRF reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of d2 and cryptate Tb-detection reagents will impair the assay quality.*
5. *Please note that this kit use a Terbium donor crypate, make sure to set up your reader for Terbium cryptate.*

CELL-BASED ASSAY MANUAL

	NEGATIVE CONTROL*	NON-STIMULATED CELLS**	STIMULATED CELLS
Step 1	Dispense 7 µL of cells into all wells.	Dispense 7 µL of cells into all wells.	Dispense 7 µL of cells into all wells.
Step 2	Add 7 µL StimB to negative control.	Add 7 µL StimB to non stimulated wells.	Add 7 µL compound (2X) in StimB to stimulated wells.
Step 3	Seal the plate and incubate for optimized period (30 min to 2 hours) at 37°C.	Seal the plate and incubate for optimized period (30 min to 2 hours) at 37°C.	Seal the plate and incubate for optimized period (30 min to 2 hours) at 37°C.
Step 4	Add 3 µL Lysis & Detection Buffer.	Add 3 µL of IP1 d2 Reagent working solution to all wells.	Add 3 µL of IP1 d2 Reagent working solution to all wells.
Step 5	Add 3 µL of IP1 Tb Cryptate Antibody working solution.	Add 3 µL of IP1 Tb Cryptate Antibody working solution.	Add 3 µL of IP1 Tb Cryptate Antibody working solution.
Step 6	Put lid on plate and incubate 1 hour at room temperature.	Put lid on plate and incubate 1 hour at room temperature.	Put lid on plate and incubate 1 hour at room temperature.
Step 7	Remove the lid and read on an HTRF compatible reader.	Remove the lid and read on an HTRF compatible reader.	Remove the lid and read on an HTRF compatible reader.

*The Negative Control wells represent the non-specific fluorescence stemming from the Cryptate.
**Data with non-stimulated cells indicate the basal cellular level of IP1.

DATA REDUCTION & INTERPRETATION

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

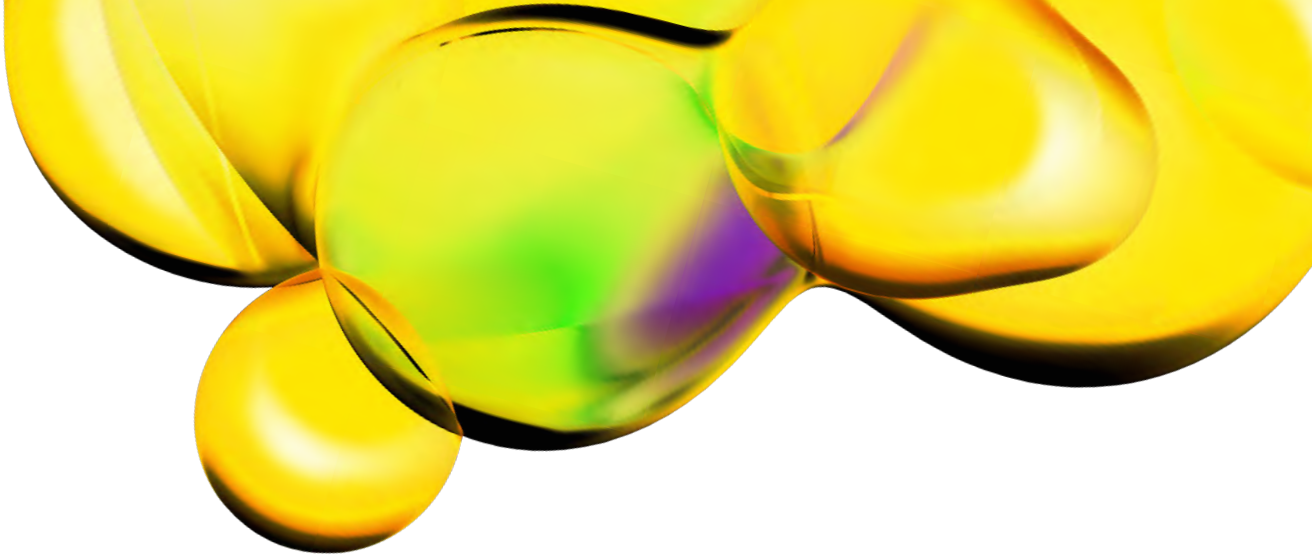
$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Plot the HTRF ratio versus compound concentrations.

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

ASSAY FLEXIBILITY AND MINIATURIZATION

When used as recommended, the kit provides sufficient reagents for 1,000; 20,000 or 100,000 tests using a 384-well low volume plate or with Revvity's HTRF 96w low volume plate in 20 µL final assay volume.
If other plate formats are used (96 half-well or 1536-well), the cell density has to be optimized depending on the surface of the well. The volume of each assay component must be proportionally adjusted to the final volume in the microplate in order to maintain the reagent concentrations as for the 20 µL final assay volume.



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

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