

For in vitro research use only - Storage temperature : 2-8°C

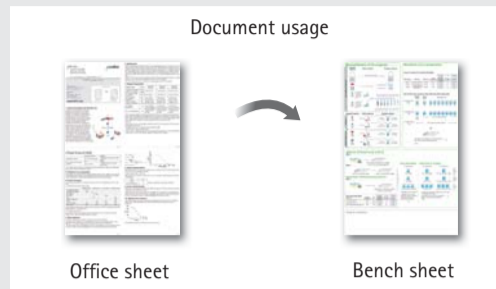
HTRF® package insert

Document reference
62P1APEX rev02 (Sept. 2008)

* 384-well low volume plate (20 µL)

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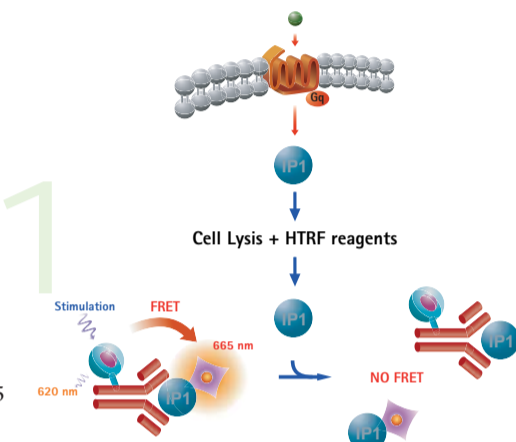
www.htrf.com



1. Assay description and intended use

Cisbio's IP-One kit is intended for the direct quantitative measurement of myo-Inositol 1 phosphate (IP1), and has been optimized for detection of IP1 directly in cultured cells. New formulation of the IP-One kit produces an increased Signal-to-Noise ratio (S/N) allowing for better cell-based assay optimization and improved screening quality. This kit is streamlined for HTS and uHTS applications.

This assay is based on a monoclonal antibody specific for IP1, labeled with Eu Cryptate. This antibody competes with native IP1 produced by cells and IP1 coupled to the dye d2. The specific signal is inversely proportional to the concentration of IP1 in the standard (Std) or in the cell lysate. As for all other HTRF® assays, data reduction using the fluorescence ratio (665 nm/620 nm) eliminates possible photophysical interference and means the assay is unaffected by the usual buffer conditions and colored compounds.



4. Reagent storage and stability

	Storage conditions	Stability
Lyophilized reagents	4°C until reconstitution	Until expiry date indicated on the labels
Stock solutions and working solutions*	4°C frozen (-20°C)	4 days 3 months. May be frozen and thawed once

*It is recommended to dispense the remaining solutions of standard and conjugates into disposable plastic vials for storage conditions after first use.

5. Standard curve preparation

The standard curve is prepared with the reagent supplied with the kit, IP1 calibrator («Cal» vial) and the stimulation buffer («StimB» vial). All information necessary for the preparation of the standard curve is detailed in the «Bench document» (please see reverse page of this document).

6. Assay procedure

The controls and standards do not require cells, but must be run in a similar plate to the rest of the assay.

6.1. Standard protocol in 20 µL final volume

	Negative control	Standard curve	Assay control	Cell-based assay
1X stimulation buffer	14 µL	-	-	-
Conjugate & lysis buffer	3 µL	-	-	-
IP1 calibrator	-	14 µL	-	-
IP1 control	-	-	14 µL	-
Cells	-	-	-	7 µL
Compound (agonist)	-	-	-	7 µL
IP1 d2	-	3 µL	3 µL	3 µL
Anti-IP1 cryptate	3 µL	3 µL	3 µL	3 µL
Total volume	20 µL	20 µL	20 µL	20 µL

After distribution of the reagents, place lid on plate, incubate 1h at room temperature (RT) and read on an HTRF® compatible reader. HTRF® signal is stable over a 24h period at RT.

6.2. Assay procedure

In the «Bench document», a detailed protocol is recommended. You can contact your technical support team to obtain additional information or support.

7. Data reduction

Results are calculated from the 665nm / 620nm ratio and expressed in Delta F.

Draw up the assay standard curve by plotting delta F% versus IP1 concentration. An example of graph is given in the figure (readout on PHERAstar Plus).

*The assay control («Ctrl» vial) validates the accuracy of the standard curve. The concentration deduced from the delta F obtained should fall into the concentration range printed on the vial label.

2. Background

GPCRs carry information within the cell via two major signaling pathways : the activation of Gs or Gi protein coupled receptors results in a variation of the cyclic AMP (cAMP) level, and the activation of Gq protein coupled receptors results in a transient increase of intracellular Ca²⁺, triggered by inositol (1,4,5) tri-phosphate (IP3). After the GPCR Gq activation, the lifetime of IP3 is very short (less than 30 sec) before being transformed into IP2 and then IP1. IP1 is accumulated in the cell when LiCl is added to the medium. After activation of the GPCR, IP1 can be precisely quantified using the IP-One assay.

The HTRF® IP-One assay has been evaluated on various GPCR models, using either stable or transient cell lines. See www.htrf.com for additional information on IP-One validation

The IP-One assay represents a new solution for Gq investigation under HTS conditions.

3. Reagent preparation (stock solutions)

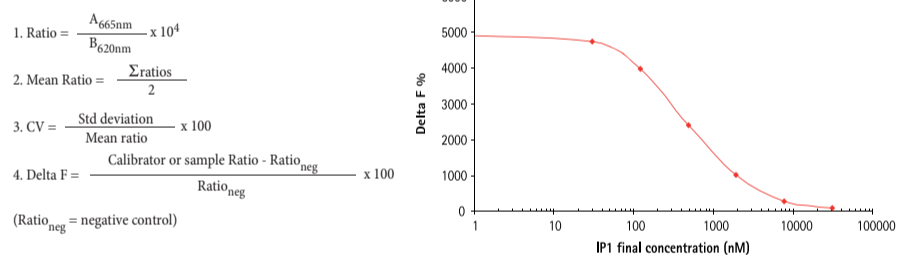
Supplied reagents	Abb*	62P1APEB 1,000 tests	62P1APEC 20,000 tests	62P1APEJ 100,000 tests
IP-One Eu conjugate & lysis buffer (ready to use solution)	Lysis Buffer	1 vial of 13 mL # 62CL3FDD	1 vial of 200 mL # 62CL4FDF	5 vials of 200 mL # 62CL4FDF
IP1 stimulation buffer (5x solution)	StimB	1 vial of 8 mL	1 vial of 100 mL	5 vials of 100 mL
IP1-d2 conjugate (lyophilized)	IP1-d2	1 vial of each (to be reconstituted with 0.5 mL of distilled water)	1 vial of each (to be reconstituted with 3 mL of distilled water)	5 vials of each (to be reconstituted with 3 mL of distilled water)
Anti-IP1 cryptate conjugate (lyophilized)	Ab-Cryp	1 vial of each (to be reconstituted with distilled water, see volume indication on label)	1 vial of each (to be reconstituted with distilled water, see volume indication on label)	1 vial of each (to be reconstituted with distilled water, see volume indication on label)
IP1 calibrator (lyophilized)	Cal	1 vial of each (to be reconstituted with distilled water, see volume indication on label)	1 vial of each (to be reconstituted with distilled water, see volume indication on label)	1 vial of each (to be reconstituted with distilled water, see volume indication on label)
IP1 control (lyophilized)	Ctrl	1 vial of each (to be reconstituted with distilled water, see volume indication on label)	1 vial of each (to be reconstituted with distilled water, see volume indication on label)	1 vial of each (to be reconstituted with distilled water, see volume indication on label)

*Abbreviation

The IP1 stimulation buffer provided with the kit (StimB 5x) has been validated on several GPCR models by Cisbio. Once diluted 1/5 in distilled water, its composition is the following one: Hepes 10 mM, CaCl₂ 1 mM, MgCl₂ 0.5 mM, KCl 4.2 mM, NaCl 146 mM, glucose 5.5 mM, LiCl 50 mM pH 7.4. It is used to dilute the IP1 standards, the agonist, antagonist or test compounds and cells.

Important:

- If you wish to use your own stimulation buffer, 50 mM of LiCl is recommended in order to prevent IP1 degradation in the cells.
- Phosphate buffer is not recommended in this stimulation buffer.
- HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Cryptate-conjugates will impair the assay quality.
- Do not mix reagents from different kits.



$$1. \text{Ratio} = \frac{A_{665nm}}{B_{620nm}} \times 10^4$$

$$2. \text{Mean Ratio} = \frac{\sum \text{Ratios}}{2}$$

$$3. \text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$$

$$4. \text{Delta F} = \frac{\text{Calibrator or sample Ratio} - \text{Ratio}_{neg}}{\text{Ratio}_{neg}} \times 100$$

(Ratio_{neg} = negative control)

8. Assay characteristics

Specificity of the assay : the assay does not show any cross reaction with Myo-inositol, PIP2, IP2, IP3, IP4 or PIP3 up to a concentration of 50 µM.

Analytical characteristics of the assay :

EC 50	S/B
500 nM (final concentration)	≈ 50

(Readout on PHERAstar Plus reader, BMG LABTECH)

Plate readout may be carried out several times over a 24 hour period without altering the performances of the assay.

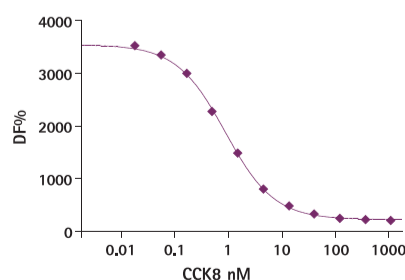
9. Assay miniaturization

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 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 according to the surface of the well. The volume of each assay component must be proportionally adjusted in order to maintain the reagent concentrations as for the 20 µL final assay volume.

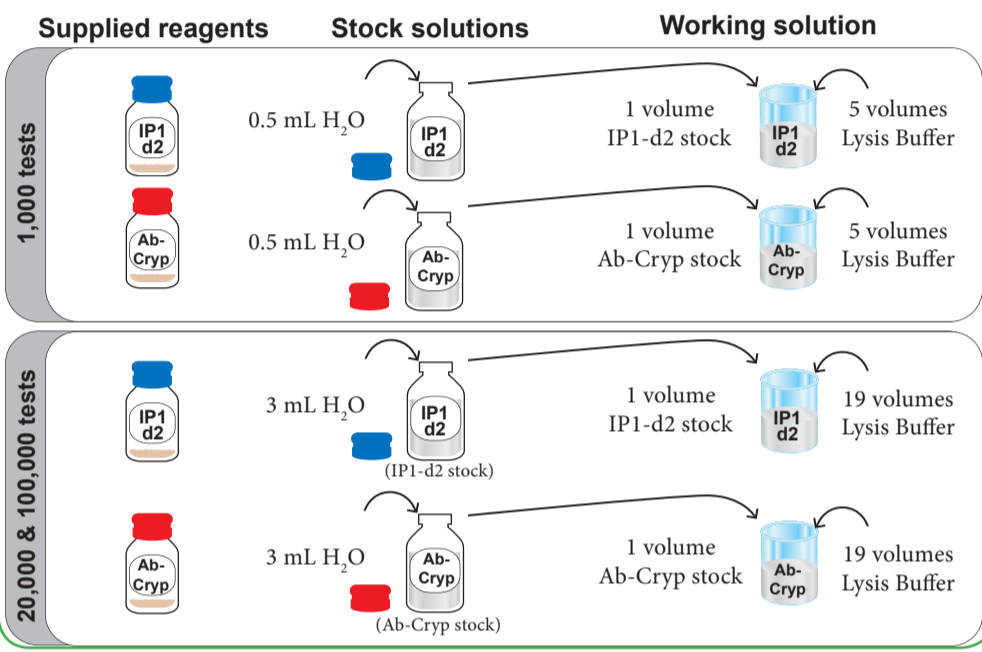
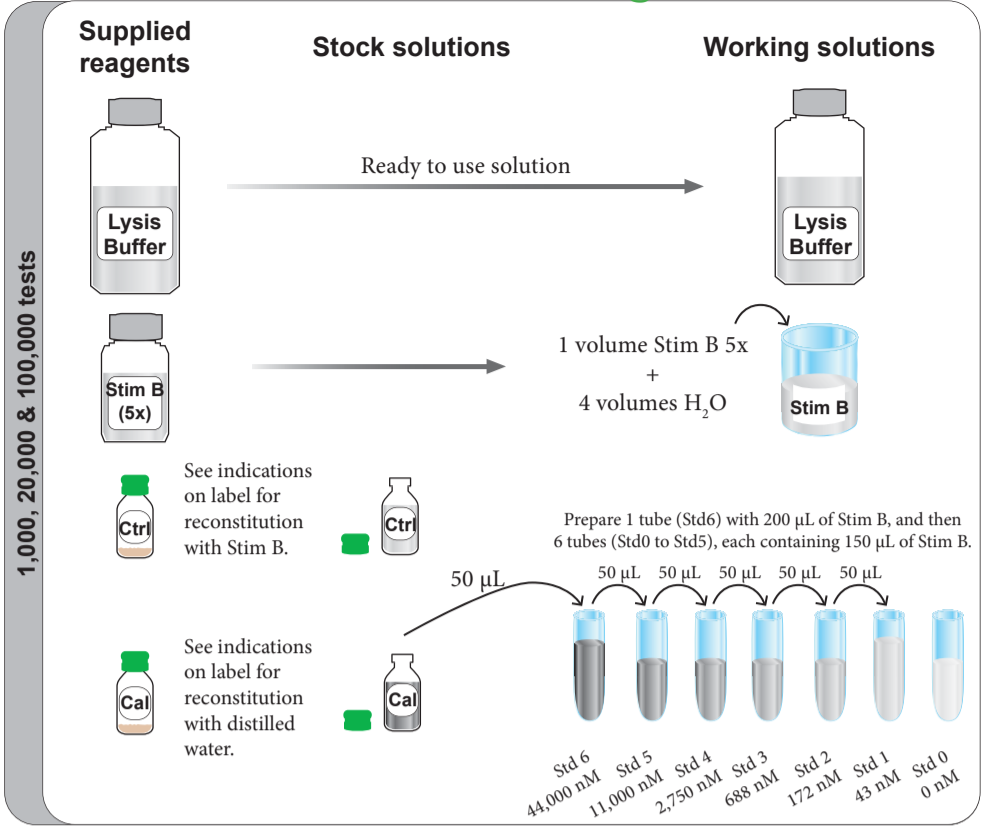
10. Agonist dose response

This section presents an agonist dose response (CCK8) obtained with the new IP-One formulation, using 1321N1 cell line expressing CCK1 (20,000 cells/well) receptor (readout performed on PHERAstar Plus).



In this experiment, the IC₅₀ of CCK8 was found to be 0.75 nM.

Reconstitution of kit reagents



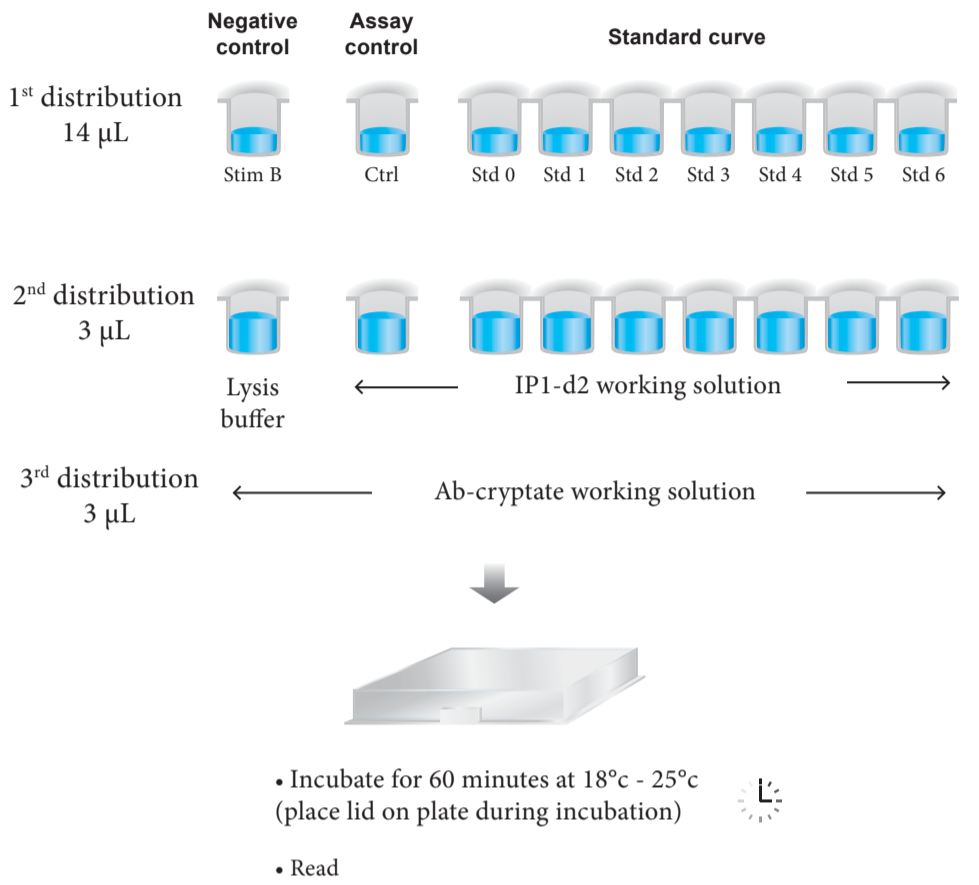
Standard curve protocol

Assay at a glance & recommended plates

Manufacturer	Plate type*	Reference	IP-One assay volume	1 st dist. Std 0 to Std 6, Ctrl or StimB	2 nd dist. IP1-d2 or Lysis Buffer	3 rd dist. Ab-Cryp
Greiner	96-well plate, white, TC	655 083	100 µL	70 µL	15 µL	15 µL
Greiner	384-well plate, white, TC	781 080	30 µL	20 µL	5 µL	5 µL
Greiner	384-well small volume plate, white, TC	784 080	20 µL	14 µL	3 µL	3 µL

Never mix the conjugate working solutions

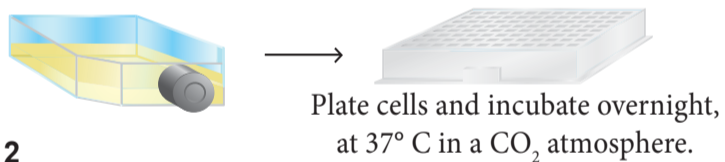
20 µL IP-One assay protocol using a 384-well small volume plate



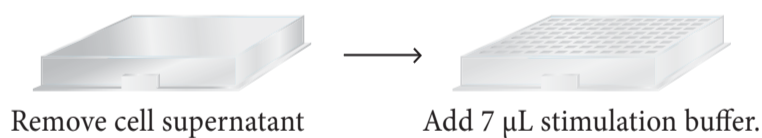
Cell-based assay protocols

Adherent cell-based assay protocol

Day 1

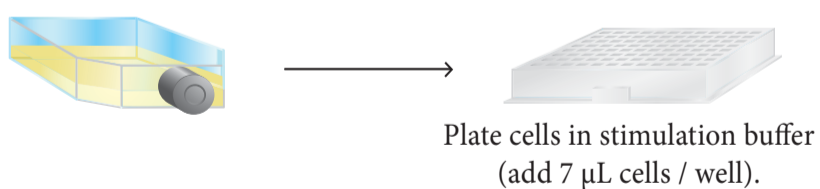


Day 2

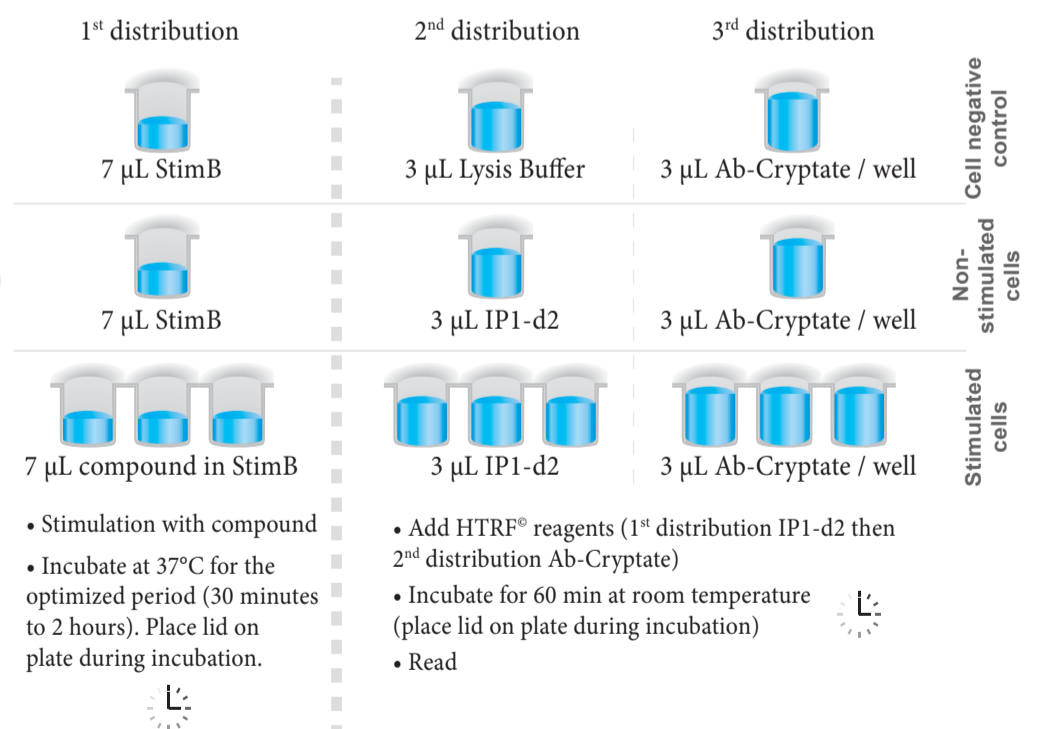


Suspension cell-based assay protocol

Day 1



Cell stimulation | Detection & readout



Recommended plates*

Plate type	IP-One assay volume	Cells	1 st dist. StimB or compound	2 nd dist. IP1-d2 or Lysis Buffer	3 rd dist. Ab-Cryp
96-well plate, white, TC	100 µL	35 µL	35 µL	15 µL	15 µL
384-well plate, white, TC	30 µL	10 µL	10 µL	5 µL	5 µL
384-well small volume plate, white, TC	20 µL	7 µL	7 µL	3 µL	3 µL

Notes & calculations