revvity

IP-One

Guide to optimizing antagonists of Gαq

PURPOSE AND BACKGROUND

Purpose

The proper optimization of assay conditions is essential to ensure you obtain the best reagent use and performance. In the case of GPCR signaling, the most suitable assay conditions depends on the GPCR coupling (Gq) efficacy as well as the pharmacological characteristics of the compound being studied (agonist or antagonist). The optimal assay conditions will also hinge on the particular cell line used for the assay. For each setup, a number of optimization steps can be implemented that will ensure the best, most accurate results are obtained.

Drawing on hundreds of data sets and cases established by Revvity's scientists over the years, this manual provides the GPCR community with the most up to date guidelines for the optimization of Gαq GPCR assays.

Background

The metabolic inositol phosphate cascade often results from the regulation of Phospholipase C-β (PLC-β) associated with the Gαq subunit of heterotrimeric G-protein. It is well known that measuring an important mediator such as D-myo-Inositol 1 -Phosphate (IP1) can be used as a surrogate for IP3. Gαq coupled GPCRs act to positively stimulate the activity of PLC-β, resulting in an increase in cellular IP1.

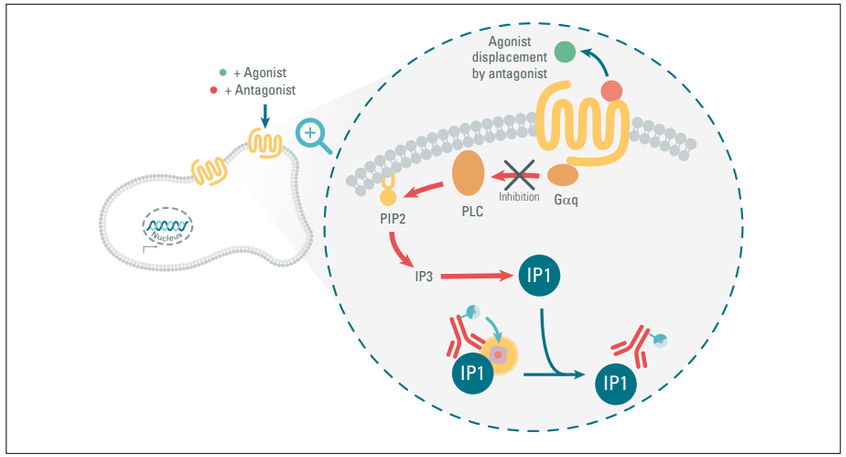
Lithium chloride (LiCl) leads to IP1 accumulation by inhibiting inositol monophosphatase, responsible for the degradation of IP1 to myo-inositol.

The IP-One kit developed by Revvity is specifically intended for the direct quantitative determination of IP1.

The assay is based on a competition between native IP1 produced by cells and IP1 labeled with the dye d2 for binding to a terbium cryptate labeled antibody. The specific signal (i.e. energy transfer signal) is inversely proportional to the concentration of IP1 in the standard or sample.

Three sizes are available to better serve your needs when quantifying IP1 accumulation in response to Gq coupled GPCR activation:

	IP-ONE GQ
1,000 tests	# 62IPAPEB
20,000 tests	# 62IPAPEC
100,000 tests	# 62IPAPEJ



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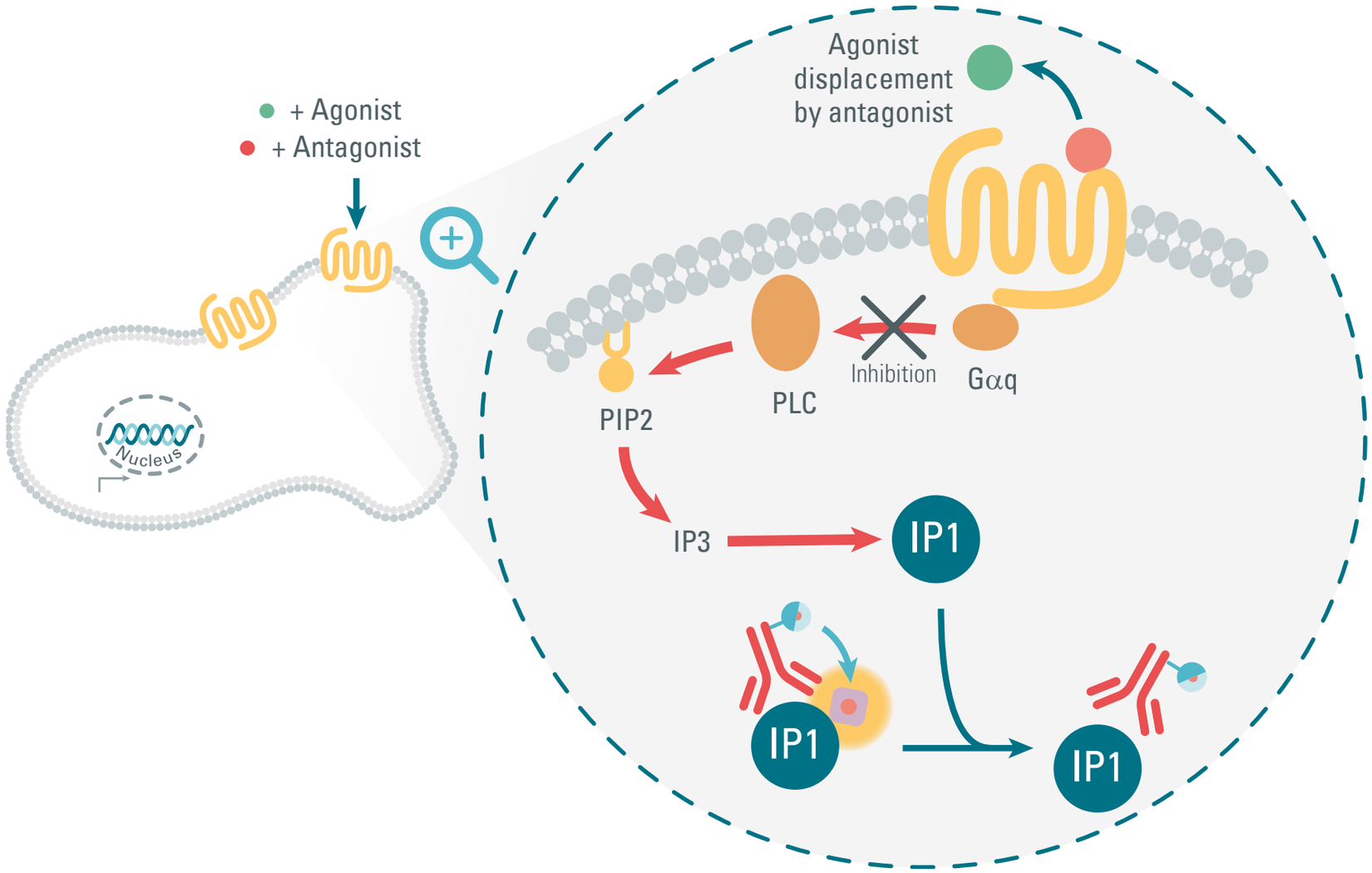

Gq pathway *Antagonists prevent the positive stimulation of the phospholipase C (PLC), resulting in a decrease in cellular IP1. After lysing the cells, the kit enables quantification of this modulation.*

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REAGENTS AND OPTIMIZATION WORKFLOW

Reagents

Reagent preparation

Stimulation buffer

The stimulation buffer included in the kits is used to prepare the cells and all the chemicals and compounds needed in the assay. It is supplemented with lithium chloride (LiCl) for its inhibitory effect on inositol monophosphatase (IMPase) to prevent degradation of IP1 into myo-inositol. Thus, IP1 can be accumulated in cells in the presence of LiCl when PLC is activated by a GPCR.

Standards

The standard vial contains a known concentration of IP1. Further dilutions of this standard can be used to establish a standard curve. Using the standard curve, the fluorescence units from the assay can be converted into concentrations of IP1. It is recommended to use the same stimulation buffer as that used for the cell based assay to quantify IP1 released by the cells.

Example: If the IP-One kit stimulation buffer was used to prepare the cells, then the same solution should also be used to reconstitute the standard and then dilute it.

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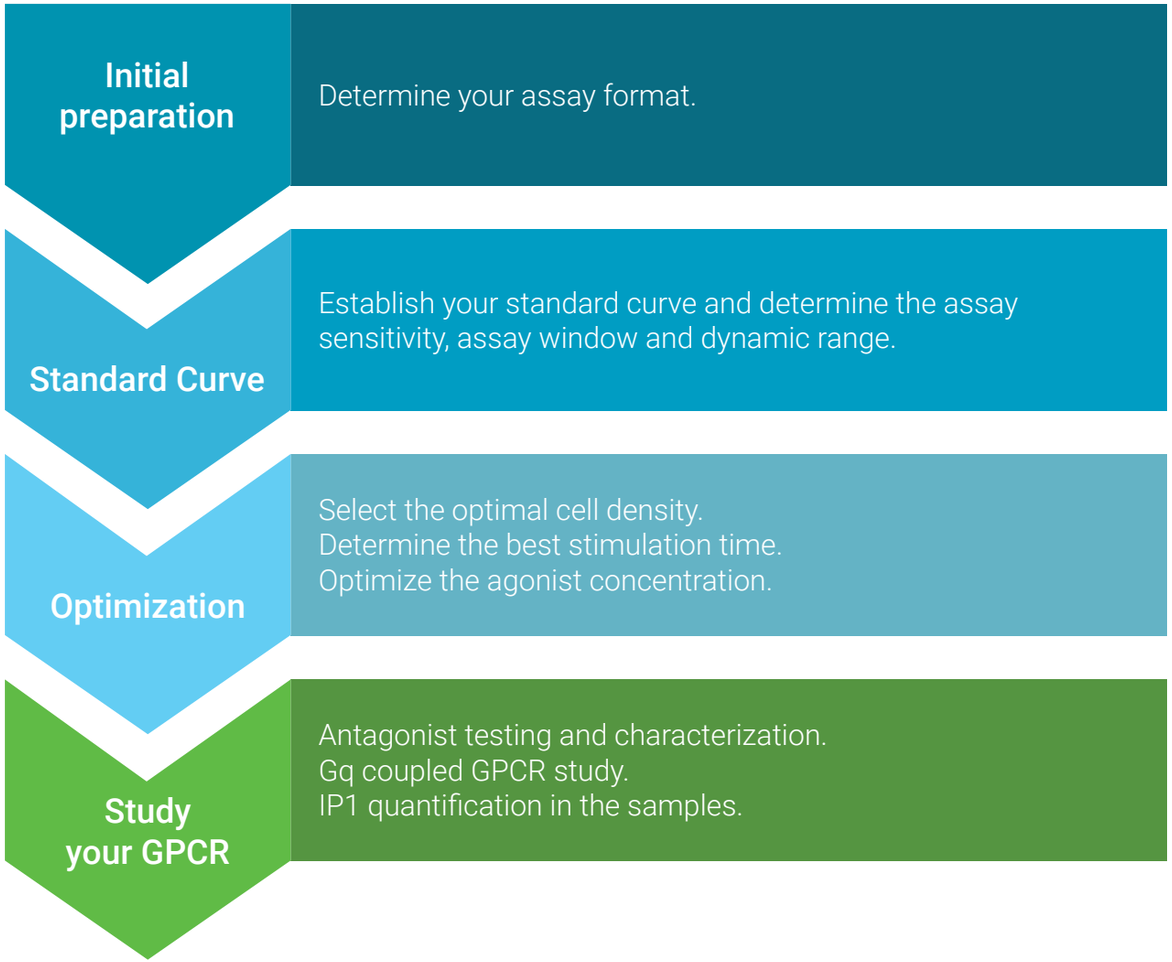
REAGENTS AND OPTIMIZATION WORKFLOW

Workflow optimization



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CELL PREPARATION
Before you begin

Cryopreserved cells have already been successfully employed in a number of IP1 studies using Revvity reagents. Advances in cell culture technologies have increasingly enabled researchers to rely on freshly cultured and collected cells. Cryopreserved vs freshly maintained cells will be discussed in this manual.

The question of adherent cells versus suspension cells is one of the first decisions a researcher must make when optimizing an IP1 assay.

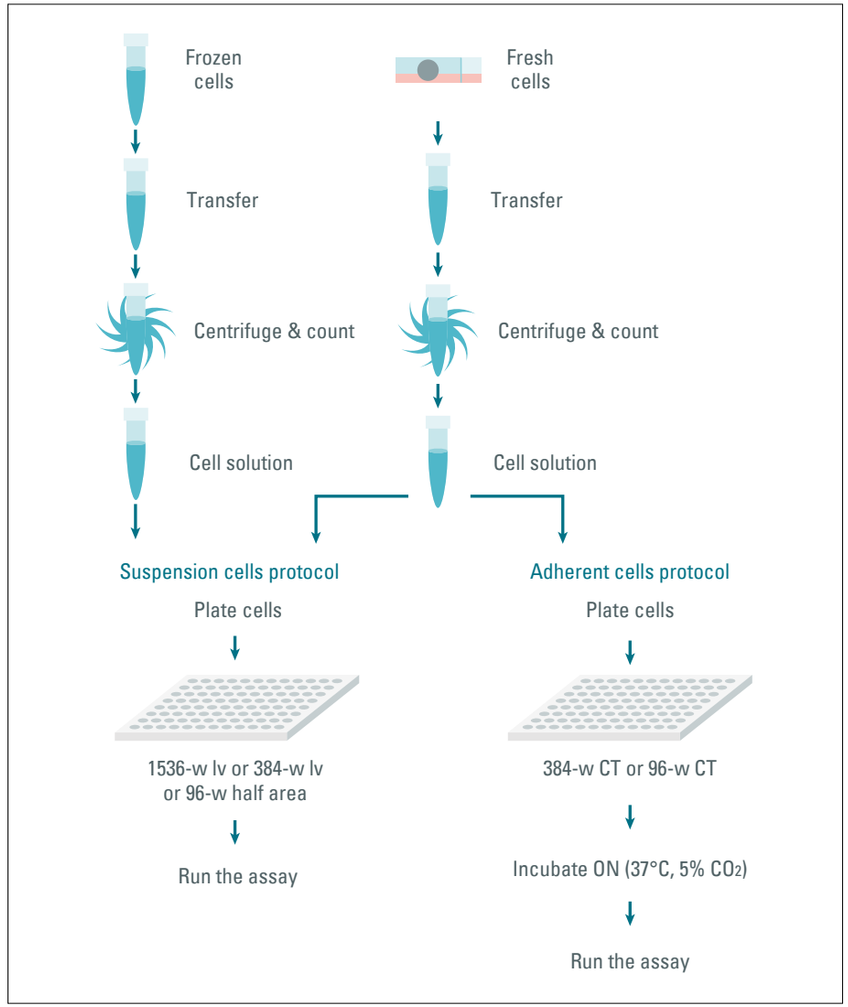
- Cells presenting an adherent phenotype are typically cultured in plates overnight prior to being assayed.
- Cells with a non-adherent phenotype are typically dispensed and tested in suspension immediately following plating.

Your decision regarding adherent cells versus suspension cells should be made on the basis of biology expectations and ease of assaying. It is possible to assay either suspension cells or adherent cells, and some guidelines for both protocols are provided below.

Finally, the IP-One assay can be performed in :

- 96-well plates
- HTRF® 96-well lv plate (ref 66PL96001)
- 96-well half area plates
- 384-well plates
- 384-well plates (low volume plates)
- 1536-well plates

The choice of plate format will depend on the preferred protocol, desired throughput, and considerations of reagent consumption.



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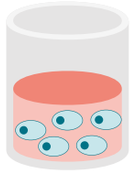
CELL PREPARATION
Freshly harvested cells

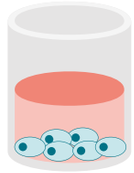
Suspension protocol (for biologically adherent cells)

- Cells from T175 cm^2 flasks should be near 80% confluency before collection.
- Remove culture medium and gently rinse flask using PBS.
- Add cell dissociating solution (5 mL for a T175 cm^2 flask), incubate the plate at 37 °C and 5% CO_2 for 5–10 min or until cells are dislodged. Add 5 mL of PBS to a final volume of 10 mL and pipette up and down until cells are homogeneously dispersed in the solution.
- Spin the cell suspension at 340 g for 3 min.
- Calculate the volume of stimulation buffer needed to re-suspend the cell pellet, achieving an optimal cell density.
- Always use the stimulation buffer, as previously discussed, when re-suspending the cells. Cell density should be optimized. Refer to the later sections of this manual for recommendations regarding cell density optimization.
- The cells are ready to be dispensed into the assay plate. Refer to table on next page for recommended dispensing volumes.
- Run the assay.



Adherent protocol

- Cells from T175 cm^2 flasks should be near 80% confluency before collection.
- Remove culture medium and gently rinse flask using PBS.
- Add cell dissociating solution (5 mL for a T175 cm^2 flask), incubate the plate at 37 °C and 5% CO_2 for 5–10 min or until cells are dislodged. Add 5 mL of PBS to a final volume of 10 mL and pipette up and down until cells are homogeneously dispersed in solution.
- Spin the cell suspension at 340 g for 3 min.
- Calculate the volume of complete growth medium needed to re-suspend the cell pellet, achieving an optimal cell density.
- Cell density should be optimized. Refer to the later sections of this manual for recommendations regarding cell density optimization. The use of cell-culture treated well-plates or Poly-d-Lysine coated plates is preferable.
- The cells are ready to be dispensed into the assay plate. Refer to table on next page for recommended dispensing volumes.
- Incubate overnight at 37°C, 5% CO_2.
- Flip the plate to remove the cell supernatant.
- Run the assay.



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CELL PREPARATION

Cryopreserved cells

- Thaw frozen cells rapidly in a water bath at 37°C until completely thawed.
- Transfer cells into a vial and add 10 mL PBS.
- Centrifuge 3 min at 340 rcf (g).
- Discard supernatant and re-suspend the pellet in stimulation buffer. Determine cell concentration and viability.
- Dilute the cells in the stimulation buffer to reach the cell concentration required for the experiment.
- Dispense the cells into the plates (refer to the table below under “suspension protocol” for the recommended volumes).
- Run the assay.

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CELL PREPARATION

Plate formats and volumes

Dispensing volumes for each step of each protocol are summarized in the table below.

	CELL BASED ASSAY OR STANDARD CURVE	 ADHERENT CELLS PROTOCOL		 SUSPENSION CELLS PROTOCOL			
Stimulation step	Plate	384-w CT white	96-w CT white	1536-w white	384-w lv white	HTRF 96-w lv plate	96-w half area white
	Cells	20 µL*	100 µL*	5 µL	10 µL	10 µL	50 µL
	Cell incubation step	Overnight at 37°C, 5% CO_2		No cell incubation			
	Compounds (3.5X) or stimulation buffer	8 µL	40 µL	2 µL	4 µL	4 µL	20 µL

* We advise the user to remove cell supernatant and to add stimulation buffer in the recommended volume.

GαQ AGONIST ASSAY OPTIMIZATION

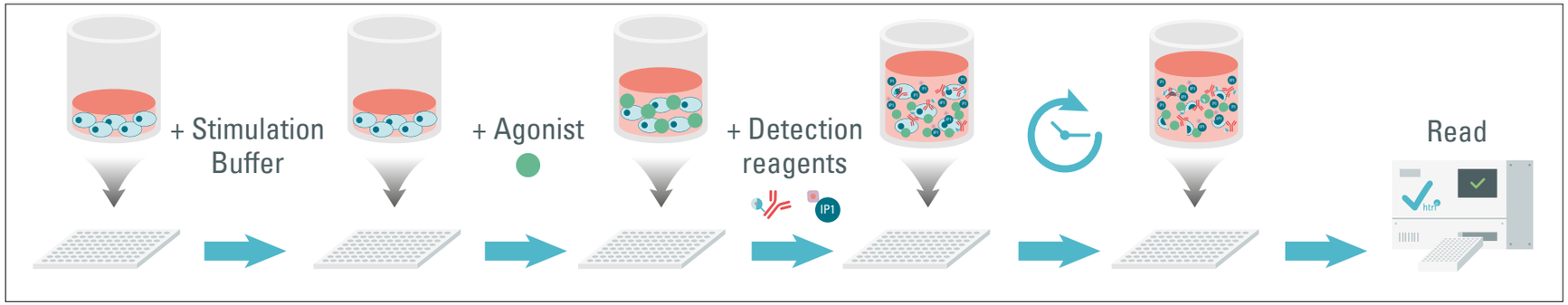
Dispensing formats

Adherent and suspension protocols have different dispensing volume requirements. A user who has opted for an adherent protocol removes the cell-culture media from the plate before proceeding with cell stimulation, and essentially starts the day with a liquid-free plate. On the other hand, a user who has selected a suspension protocol starts the day with cells in suspension in a volume of liquid.

For each protocol, the recommended dispensing volumes must be reviewed.

Untreated cells, where the agonist is substituted for an equivalent volume of buffer, are a useful control. From this control, the basal levels of IP1 production can be assessed.

Suspension protocol



CELL BASED ASSAY		SUSPENSION CELLS PROTOCOL			
	Plate	1536-w white	384-w lv white	HTRF 96-w lv white	96-w Half area white
Stimulation step	Cells	5 µL	10 µL	10 µL	50 µL
	Stimulation buffer 1X	1 µl	2 µl	2 µl	10 µL
	Agonist (7X) or buffer	1 µL	2 µL	2 µL	10 µL
	Incubate agonist with cells	Necessary time at 37°C			
Detection step	IP1-d2	1.5 µL	3 µL	3 µL	15 µL
	Anti-IP1-Cryptate	1.5 µL	3 µL	3 µL	15 µL
Final assay volume		**10 µL**	**20 µL**	**20 µL**	**100 µL**

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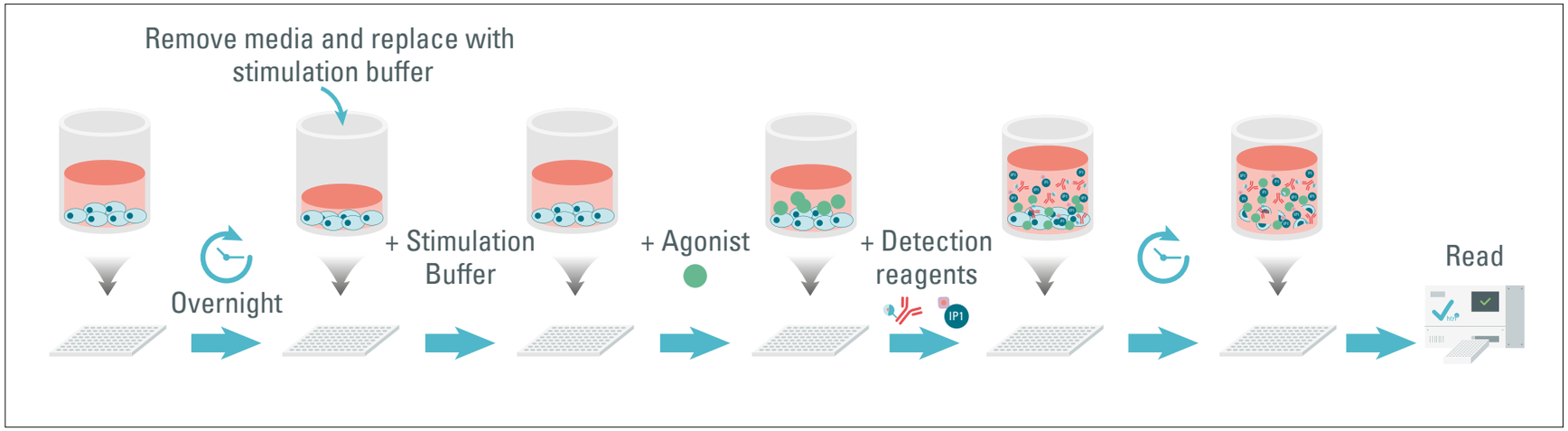
GαQ AGONIST ASSAY OPTIMIZATION

Dispensing formats

Adherent protocol



	CELL BASED ASSAY	ADHERENT CELLS PROTOCOL	
	Plate	384-w CT white	96-w CT white
Stimulation step	Cells	20 µL*	100 µL*
	Cell incubation step	overnight at 37°C, 5% CO_2	
	Stimulation buffer 1X	4 µL	20 µL
	Agonist (7X) or buffer	4 µL	20 µL
	Incubate agonist with cells	Necessary time at 37°C	
Detection step	IP1-d2	6 µL	30 µL
	Anti-IP1-Cryptate	6 µL	30 µL
Final assay volume		**40 µL**	**200 µL**

* We advise the user to remove cell supernatant and to add stimulation buffer in the recommended volume.

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GαQ AGONIST ASSAY OPTIMIZATION

Dispensing formats

Standard curve protocol

The standard curve enables quantification of the IP1 production into cells. It is recommended to use the same stimulation buffer as that used for the cell based assay to quantify IP1 released by the cells.

		ADHERENT CELLS PROTOCOL		SUSPENSION CELLS PROTOCOL			
	Plate	384-w CT white	96-w CT white	1536-w white	384-w lv white	HTRF 96-w lv plate	96-w half area white
	Standard	28 µL	140 µL	7 µL	14 µL	14 µL	70 µL
Detection step	IP1-d2	6 µL	30 µL	1.5 µL	3 µL	3 µL	15 µL
	Anti-IP1-Cryptate	6 µL	30 µL	1.5 µL	3 µL	3 µL	15 µL

Choosing between the adherent or suspension protocols depends on the biological model and the receptor studied. The adherent protocol could be more physiologically relevant in some cases, while the suspension protocol is more rapid and miniaturizable. It is possible to run a simple side by side experiment with your internal cell model on a reference agonist to test the two protocols, and then choose the protocol which is best adapted to your experiments. As an example, we describe hereafter a side by side comparison between the suspension cells protocol and the adherent cells protocol with a Gq receptor cellular model: the muscarinic M1 receptor expressing CHO cells (biologically adherent). The example demonstrates IP1 detection ability using suspension and adherent cells protocols with the same potency for the well-known M1 receptor agonist, Carbachol.

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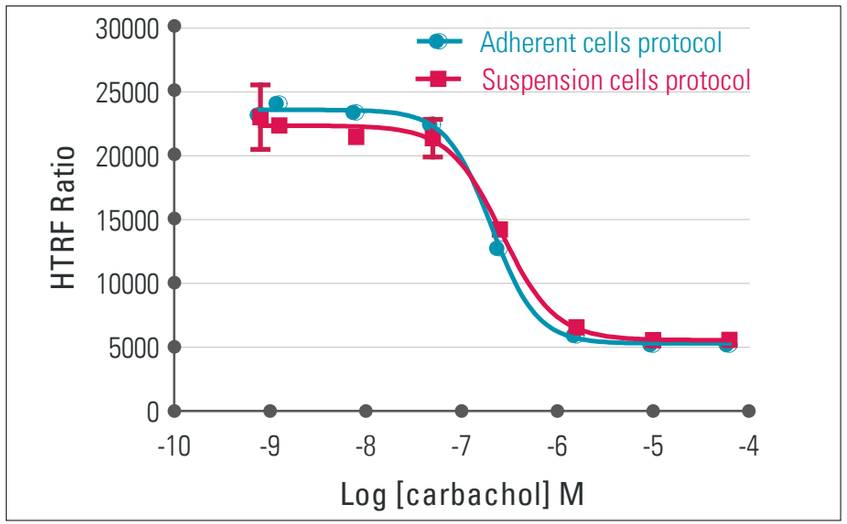
GαQ AGONIST ASSAY OPTIMIZATION

Dispensing formats

The following table describes the main protocol used for the experiment:

	CELL BASED ASSAY	ADHERENT CELLS PROTOCOL	SUSPENSION CELLS PROTOCOL
	Plate	384-w CT white	384-w lv
Stimulation step	Cells	20 µL*	10 µL
	Incubation	Overnight at 37°C, 5% CO_2	No incubation
	Stimulation buffer (1X)	4 µL	2 µL
	Carbachol	4 µL	2 µL
	Incubate agonist	45 min at 37°C	45 min at 37°C
Detection step	IP1-d2	6 µL	3 µL
	Anti-IP1-Cryptate	6 µL	3 µL
Final assay volume		**40 µL**	**20 µL**

* for adherent cells protocol, cell supernatant was removed and replaced by stimulation buffer in the recommended volume.



TYPE OF PROTOCOL	ADHERENT	SUSPENSION
S/B	4.4	4.1
EC_{50} Carbachol (µM)	0.21	0.26

Side by side comparison between suspension cells protocol and adherent cells protocol: CHO cells expressing the muscarinic M1 receptor were dispensed at a density of 20,000 cells /well. Serial dilutions of carbachol, an M1R agonist, were added and incubated for 45 min. The HTRF Ratio data were plotted to trace the sigmoidal dose response, and to calculate the EC_{50} (potency) of the compound.

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GαQ AGONIST ASSAY OPTIMIZATION

Standard curve

The standard curve will be extremely useful in assessing the assay sensitivity (IC_{50}), the maximum assay window, and the assay dynamic range (IC_{10}-IC_{90})

Establishing the standard curve

Refer to the most recent package insert accompanying your test kit for complete instructions on standard reconstitution and dilutions. In all cases, the stimulation buffer must be used to reconstitute the standard and dilute it.

Curve analysis

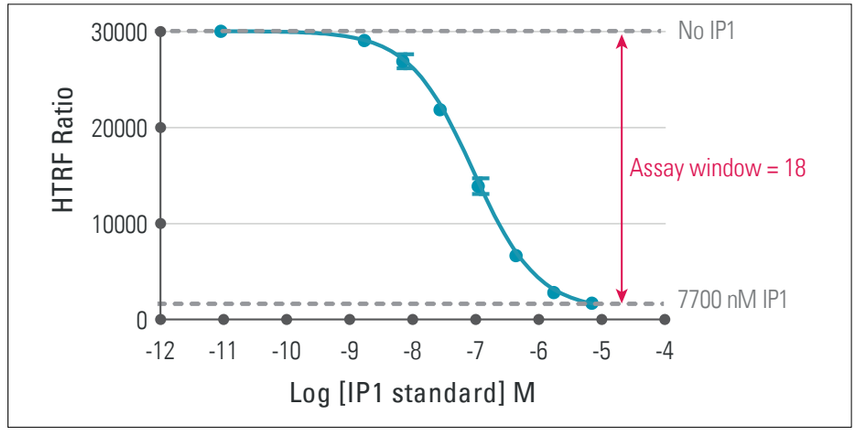
The curve is analyzed by plotting the Ratio as a function of the [IP1] of the different standards expressed as a logarithm. Graphpad Prism can be used to analyze the curve. The preferred fit is the log(inhibitor) vs. response - Variable slope. Several parameters can be derived from the fit:

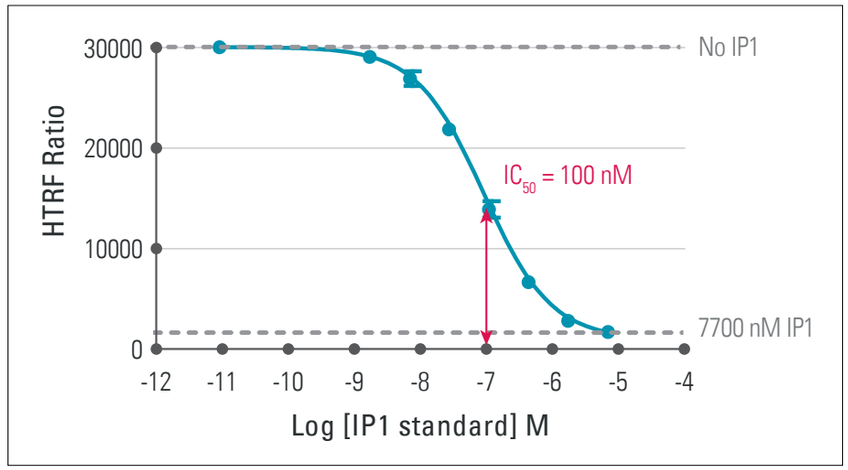
- Maximum assay window & IC50

The standard curve assay window is obtained by dividing the Ratio obtained in the absence of IP1 by the Ratio obtained by testing the highest concentrated IP1 standard. It represents the highest achievable assay window which can be obtained from the system, and will always be higher than the assay window recorded when cells are added to the system.

$$\text{Assay window} = \frac{\text{Max HTRF Ratio (No IP1 Std)}}{\text{Min HTRF Ratio (Max IP1 Std)}}$$

The IC_{50} value is the concentration of IP1 at which 50% of the initial signal is lost. In other words at IC_{50}, the concentration of IP1 present in the assay is such that half the initial FRET produced by the IP1-d2/Anti-IP1-Tb cryptate has been competed off. The IC_{50} is a measurement of how efficient and responsive the system is at recording changes in IP1 concentrations.





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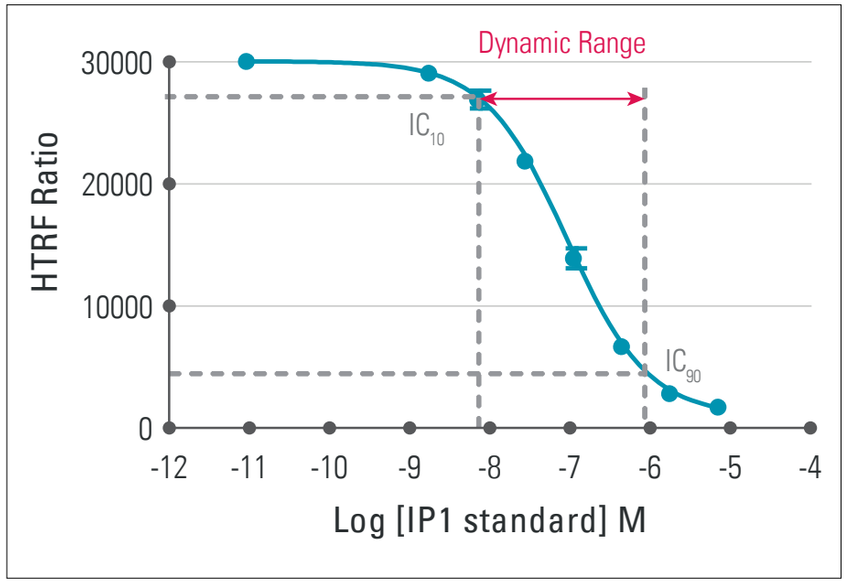
GαQ AGONIST ASSAY OPTIMIZATION

Standard curve

- Dynamic range

The IC_{10} - IC_{90} determines the assay dynamic range. This range plays a central role in assay optimization. As will be explained later in this manual, it is of primary importance that the signal recorded when assaying cells lies within the assay linear range of the standard curve. Failure to restrict Ratio values to the assay dynamic range will result in significant potency inaccuracies when assaying compounds on cells.

Using GraphPad Prism, determine the standard curve IC_{10} and IC_{90} values, and record the corresponding Ratio and [IP1].



The assay dynamic range indicates the concentration of IP1 the assay can accurately quantify. Samples with IP1 concentrations above IC_{90} or below IC_{10} cannot be reliably estimated. Similarly, samples with Ratio values below IC_{90} and above IC_{10} cannot be reliably quantified.

LINEAR RANGE	HTRF RATIO	[IP1] nM
IC_{10}	27180	7
IC_{90}	4500	916

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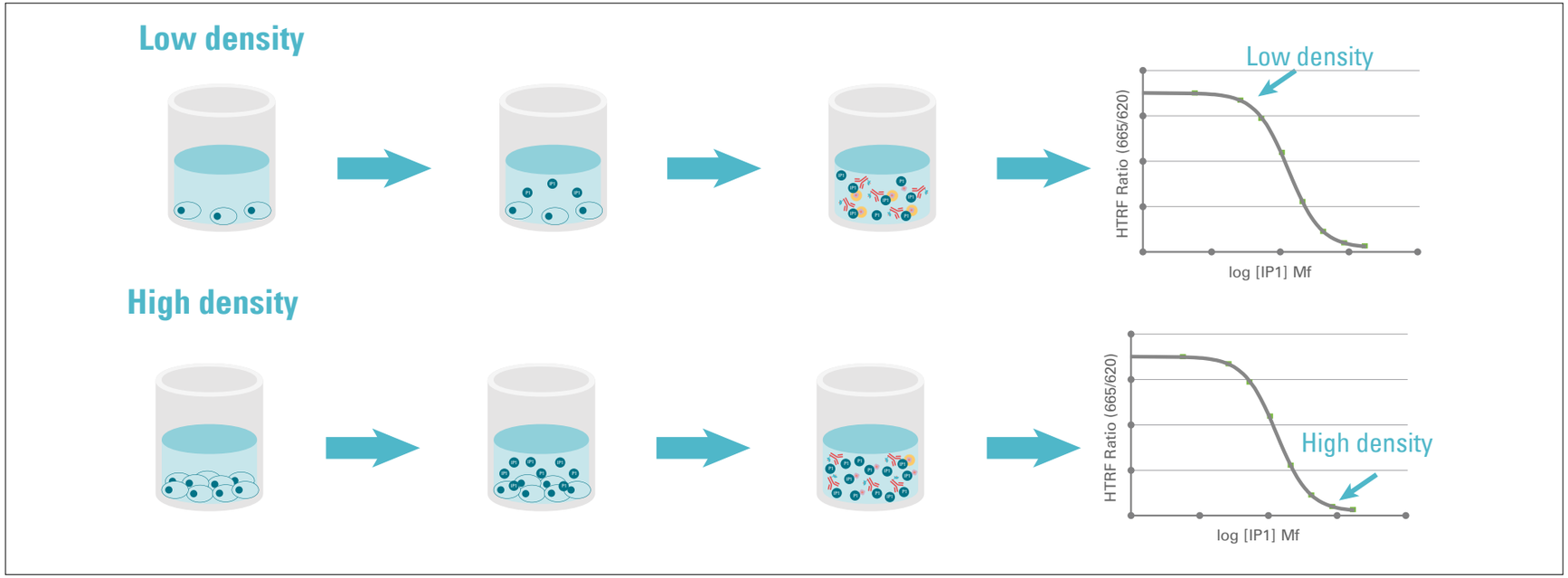
GαQ AGONIST ASSAY OPTIMIZATION

Cell density

Optimizing the cell density is the key to obtaining the best possible results. Too low a cell density may not result in enough IP1 production for the assay to record. Too high a cell density may give a dramatic decrease in the assay window.



The assay performance must be evaluated for a range of cell densities. The preferred optimization method is to assay the concentration-effect of a full agonist over a range of cell densities. Note that a full dose response is preferred to assaying a single concentration of agonist.

Organizing the standard curve graph and the cell density graph side by side reveals at a glance which cell densities are within the assay range. Cell densities "in range" are those for which the reference agonist dose response lies within IC_{90}-IC_{10} of the standard curve.

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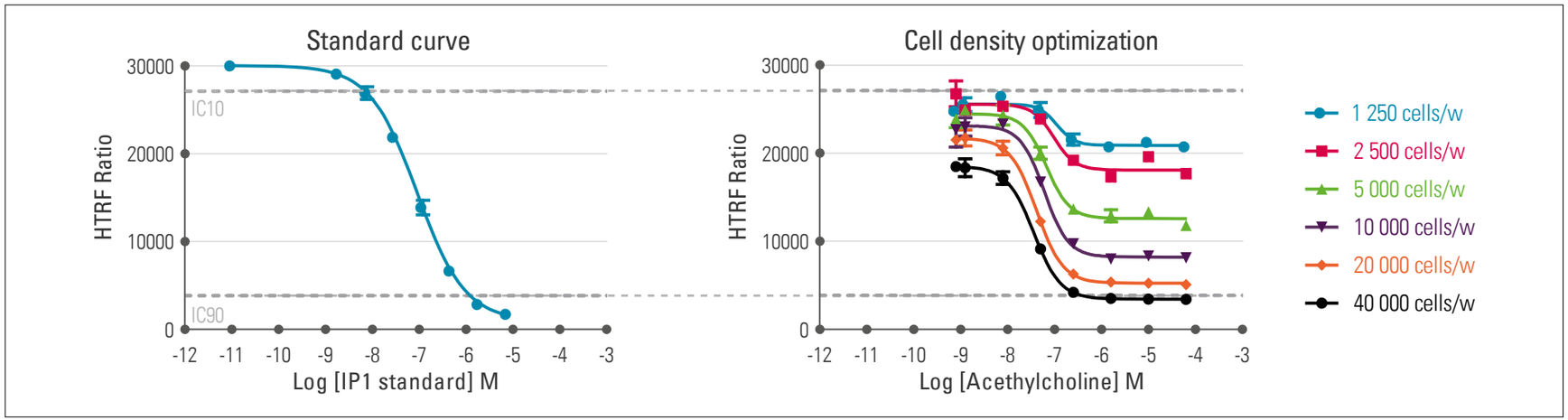
Cell density



CELLS	1,250 CELLS/W	2,500 CELLS/W	5,000 CELLS/W	10,000 CELLS/W	20,000 CELLS/W	40,000 CELLS/W
Assay window	1.2	1.5	2.0	2.8	4.2	5.5
EC_{50} Acetylcholine (nM)	100	97	70	66	51	42

Dose response curves for CHO cells stably expressing the Muscarinic M1 receptor, stimulated with Acetylcholine, a reference agonist. Cells were dispensed into a 384-lv white plate and stimulated 45 min at 37 °C. 3 µl of each detection reagent were then added for detection. Reading was done with a Pherastar lamp reader after 1h incubation time at 22 °C. A cell density of 20,000 cells per well is optimal. This density maximizes the assay window while ensuring the signal remains within the standard curve dynamic range. The highlighted cell densities in the table (in light blue) represent the cell densities for which the quantification of IP1 is out of the dynamic range of the assay.

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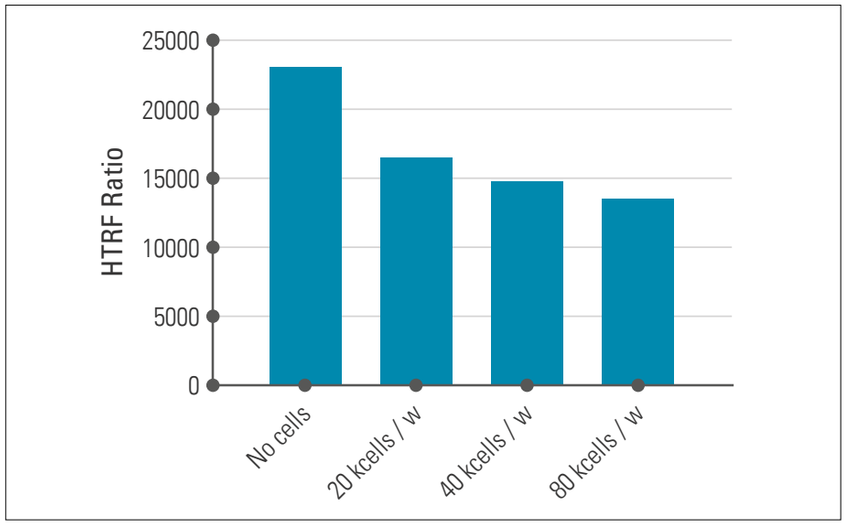
GαQ AGONIST ASSAY OPTIMIZATION

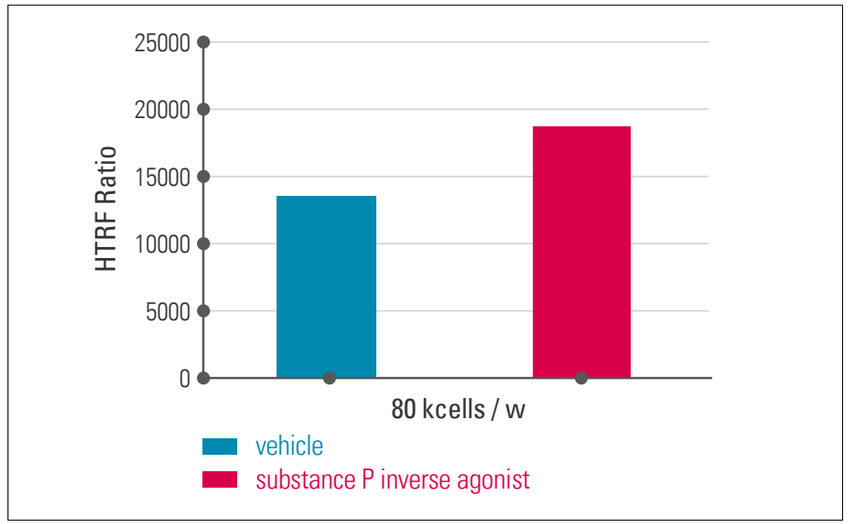
Constitutive activity

Constitutive activity is observed at both low and high GPCR expression levels and has been observed for Gαs, Gαi, and Gαq coupled GPCRs. For Gαq coupled GPCRs, constitutive activity is the production of IP1 in the absence of any agonist stimulation. High constitutive activity negatively impacts the assay window.

Fortunately, the extent to which constitutive activity reduces an assay window can readily be assessed by following the steps described in "Cell density optimization".

In this example, increased cell densities lead to a decrease in Ratio, suggesting an increase in IP1 assayed. Since this occurs in the absence of agonist stimulation, it can be concluded that the receptor constitutively produced IP1.





Highlighting the constitutive activity of the Gq coupled receptors in HEK293 cells overexpressing the GHS-R1a receptor. The HTRF Ratio decreases when the cell density increases, a consequence of the cells being constitutively active. In this example, a 1.7 fold loss in assay signal is recorded. The constitutive activity was reversed after cell treatment with the substance P inverse agonist.

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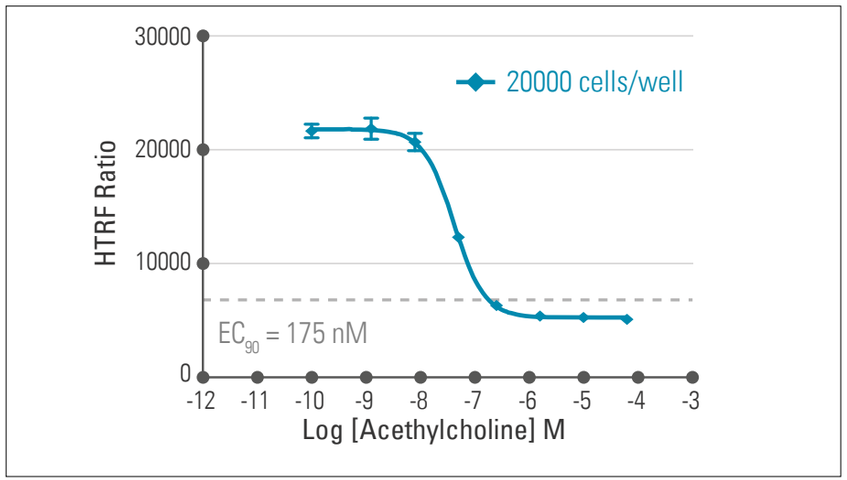
GαQ AGONIST ASSAY OPTIMIZATION

Optimized conditions for agonist testing

The optimal cell density is 20,000 cells / well. This cell density maximizes the assay window, producing IP1 quantities within the boundaries of the standard curve. The graph corresponding to the optimal density of 20,000 cells per well is shown here.

Next identify the agonist EC_{90}. The EC_{90} is the concentration of agonist that leads to a 90% reduction in signal. In this case, the EC_{90} of Acetylcholine is 175 nM .



Dose-response curve for CHO-M1 stable cells stimulated with Acetylcholine, a muscarinic M1 receptor and reference agonist: The agonistt EC_{90} will be the concentration producing 90% of the maximum IP1 response and the concentration of agonist to be tested in the presence of antagonist.

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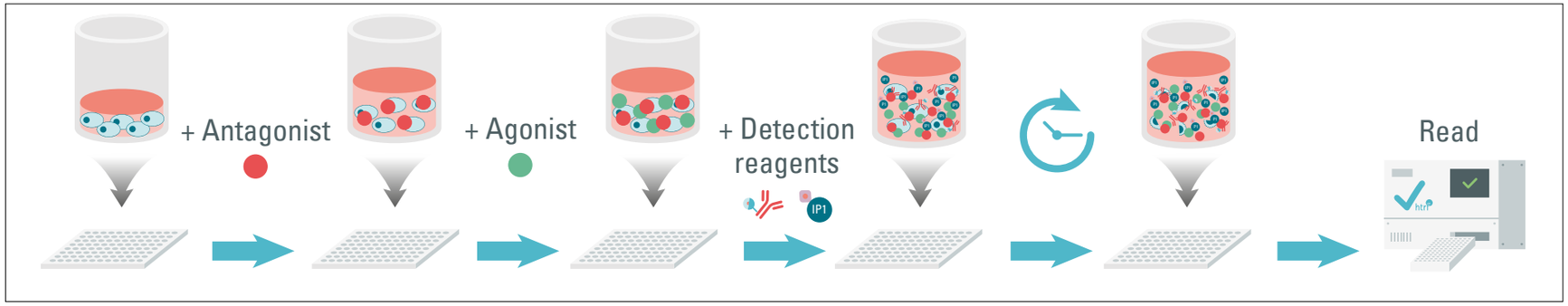
GαQ ANTAGONIST ASSAY OPTIMIZATION

Dispensing formats

Antagonist dilutions are prepared 7x the desired final concentration and added before the agonist at EC_{90}. Untreated cells, where the antagonist is substituted for an equivalent volume of buffer, are a useful control. From the control, the level of IP1 upon stimulation with the agonist at EC90 can be assessed.

Suspension protocol



	CELL BASED ASSAY	SUSPENSION CELLS PROTOCOL			
	Plate	1536-w white	384-w lv white	HTRF 96-w lv white	96-w Half area white
Stimulation step	Cells	5 µL	10 µL	10 µL	50 µL
	Antagonist (7X) or buffer	1 µl	2 µl	2 µl	10 µL
	Incubate antagonist with cells	Necessary time at 37°C			
	Agonist (7X)	1 µL	2 µL	2 µL	10 µL
	Incubate with cells	Necessary time at 37°C			
Detection step	IP1-d2	1.5 µL	3 µL	3 µL	15 µL
	Anti-IP1-Cryptate	1.5 µL	3 µL	3 µL	15 µL
Final assay volume		**10 µL**	**20 µL**	**20 µL**	**100 µL**

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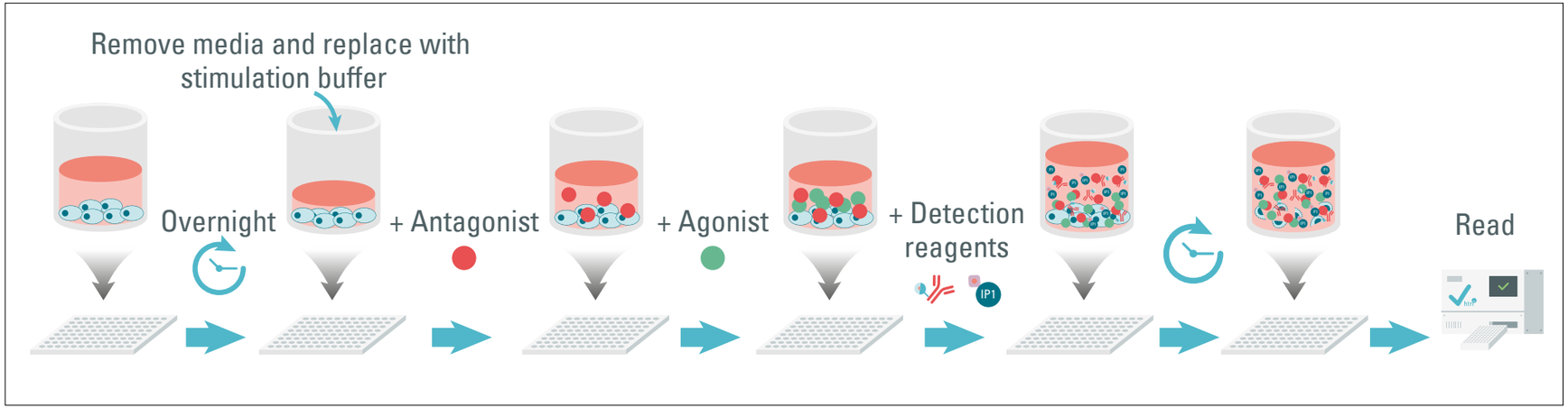
GαQ ANTAGONIST ASSAY OPTIMIZATION

Dispensing formats

Adherent protocol



	CELL BASED ASSAY	ADHERENT CELLS PROTOCOL	
	Plate	384-w CT white	96-w CT white
Stimulation step	Cells	20 µL	100 µL
	Cell incubation step	overnight at 37°C, 5% CO_2	
	Antagonist (7X) or buffer	4 µL	20 µL
	Incubate antagonist with cells	Necessary time at 37°C	
	Agonist (7X)	4 µL	20 µL
	Incubate with cells	Necessary time at 37°C	
Detection step	IP1-d2	6 µL	30 µL
	Anti-IP1-Cryptate	6 µL	30 µL
Final assay volume		**40 µL**	**200 µL**

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GαQ ANTAGONIST ASSAY OPTIMIZATION

Dispensing formats

Standard curve protocol

The standard curve enables quantification of the IP1 production into cells. It is recommended to use the same stimulation buffer as that used for the cell based assay to quantify IP1 released by the cells.

		ADHERENT CELLS PROTOCOL		SUSPENSION CELLS PROTOCOL			
	Plate	384-w CT white	96-w CT white	1536-w white	384-w lv white	HTRF 96-w lv plate	96-w half area white
	Standard	28 µL	140 µL	7 µL	14 µL	14 µL	70 µL
Detection step	IP1-d2	6 µL	30 µL	1.5 µL	3 µL	3 µL	15 µL
	Anti-IP1-Cryptate	6 µL	30 µL	1.5 µL	3 µL	3 µL	15 µL

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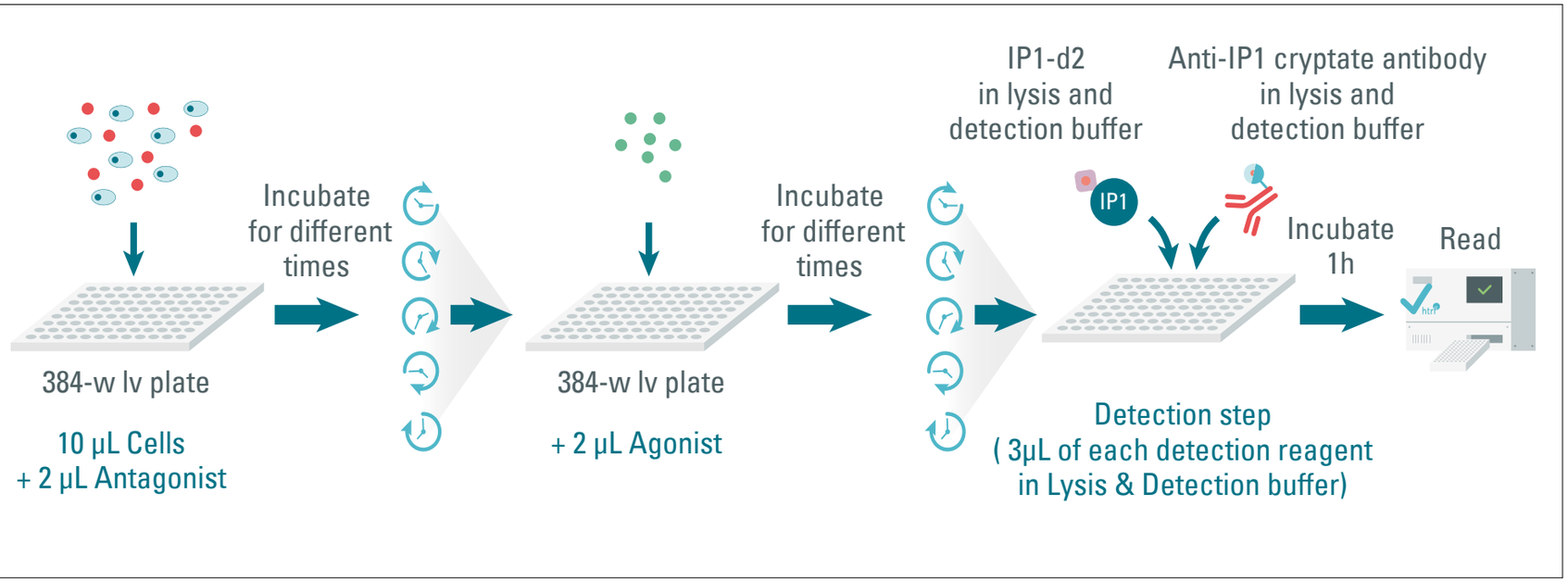
GαQ ANTAGONIST ASSAY OPTIMIZATION

Stimulation time

The antagonist must be fully pre-equilbriated prior to adding the agonist. We suggest testing different antagonist incubation times.

The agonist must also be fully equilibriated prior to adding the detection reagents. We suggest optimizing the agonist incubation time as well.



Note that first time users may proceed directly to optimizing their agonist stimulation time in the absence of the antagonist. Cells in the presence of the pre-equilibrated antagonist are stimulated with the agonist for the optimal duration previously identified. This convenient method is only recommended when a rapid answer regarding the antagonistic nature of the compound is sought. For accurate pharmacology, proceed to optimizing the agonist stimulation time in the presence of the pre-equilibrated antagonist.

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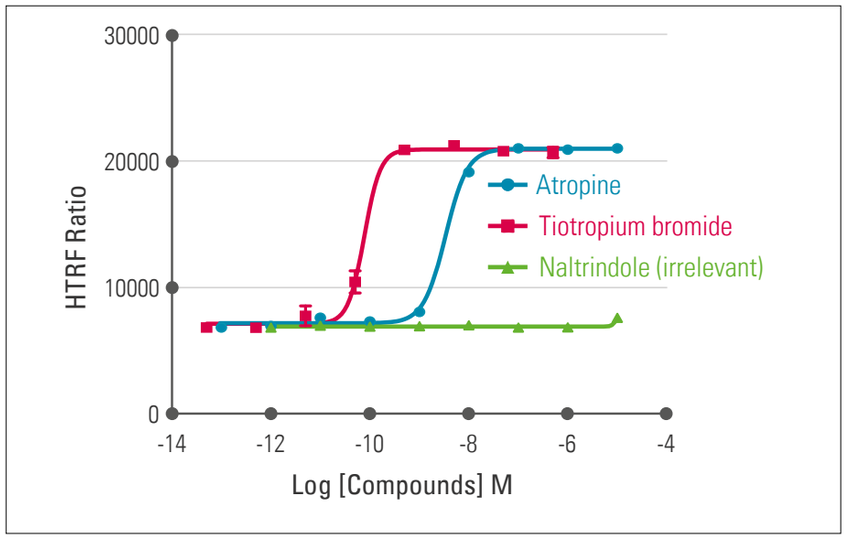
GαQ ANTAGONIST ASSAY OPTIMIZATION

Optimized conditions for antagonist testing

In this example, an EC_{90} concentration of acetylcholine was used to stimulate cells pre-incubated with either Atropine or Tiotropium bromide, 2 known antagonists of the Muscarinic M1 receptor.



	ATROPINE	TIOTROPIUM BROMIDE	NALTRINDOLE (IRRELEVANT)
IC_{50} (nM)	4	0.08	none

Antagonist screening (muscarinic M1 receptor): CHO cells stably expressing the muscarinic M1 receptor were dispensed at 20,000 cells/well in a 384 sv well plate. Test compounds was added and incubated 45 min at 37°C. The agonist acetylcholine was added at 175 nM (EC_{90}), and incubated 45 min at 37°C. Dose-response curves were plotted to obtain the IC_{50} values of the antagonists. Muscarinic M1 receptor antagonist tiotropium is 50 fold more potent than atropine, while naltrindole was used as a negative control.

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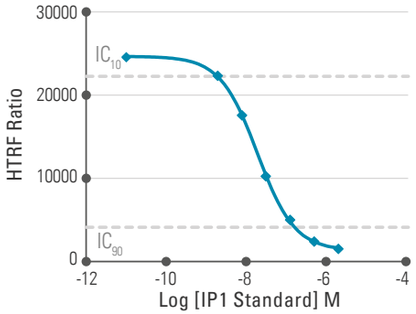
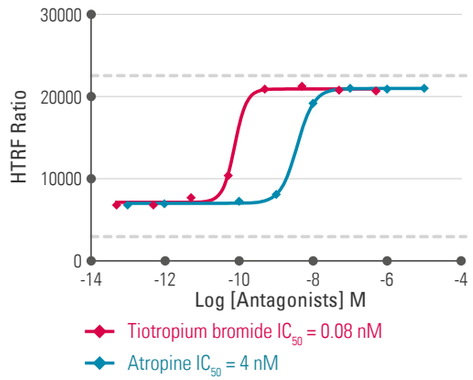
DATA REDUCTION AND ANALYSIS

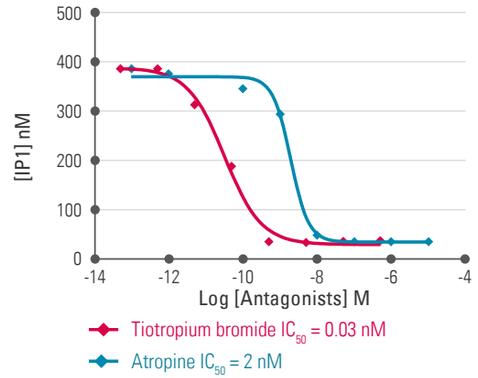
Data reduction and analysis

The fluorescence resonance energy transfer ratio (665 nm/620 nm) measurementsshould be converted into IP1 concentration-response curves to determine the IC_{50} value of the compound tested. Conversion from Ratio to IP1 concentration can be obtained by using the standard curve. Note that this conversion will produce a stimulation sigmoid dose response, as would be expected for an antagonist.





Data conversion



STANDARD CURVE	
Log [IP1] M	HTRF Ratio
-10.00	28513
-8.72	27955
-8.11	26734
-7.52	21586
-6.92	13035
-6.32	6248
-5.72	2829
-5.11	1702

CELL BASED			
Tiotropium bromide		Atropine	
Log [ ] M	HTRF Ratio	Log [ ] M	HTRF Ratio
-13.30	6832	-13.00	6832
-12.30	6830	-12.00	6960
-11.30	7766	-10.00	7318
-10.30	10460	-9.00	8079
-9.30	20937	-8.00	19157
-8.30	21291	-7.00	21051
-7.30	20823	-6.00	20946
-6.30	20719	-5.00	21051

CELL BASED			
Tiotropium bromide		Atropine	
Log [ ] M	[IP1] nM	Log [ ] M	[IP1] nM
-13.30	387.8	-13.00	386.6
-12.30	388	-12.00	376.2
-11.30	313.9	-10.00	346.4
-10.30	188.2	-9.00	293.9
-9.30	34.8	-8.00	47.7
-8.30	32.6	-7.00	34.1
-7.30	35.6	-6.00	34.8
-6.30	36.3	-5.00	34.1

Data conversion into IP1 concentration. CHO cells overexpressing the muscarinic M1 receptor were treated with Atropine (antagonist) and with Tiotropium bromide (insurmountable antagonist). In parallel, an IP1 standard curve was generated. The antagonist dose response curves initially expressed in HTRF Ratio were plotted against the standard curve to extrapolate the corresponding IP1 concentration. A new sigmoidal dose response curve was then fitted using log [compound] against [IP1], using appropriate software (e.g. Graphpad Prism). The potency of the compounds was determined and the ranking remains the same after conversion.

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DETECTION

Choosing the Right Plate Reader

Choosing the suitable microplate reader ensures you'll get an optimal readout. A multimodal reader gives your research the ideal flexibility and expands the field of possibilities by measuring a wide range of technologies.

Revvity offers multimode plate readers that are certified for use with HTRF technology. Certification involves rigorous testing of plate reader with HTRF technology to ensure optimal readout. HTRF assays are also compatible with multimode readers from other providers as well.

EnVision® Nexus™

Provides lighting speed and superior sensitivity across all established detection technologies with advanced options for ultimate performance. It is the next generation of high-throughput screening, ideal for your most demanding assays.

VICTOR® Nivo™

Packs all the latest major detection technologies into the industry's smallest benchtop footprint. The perfect microplate reader for everyday biochemical and cell-based assays.

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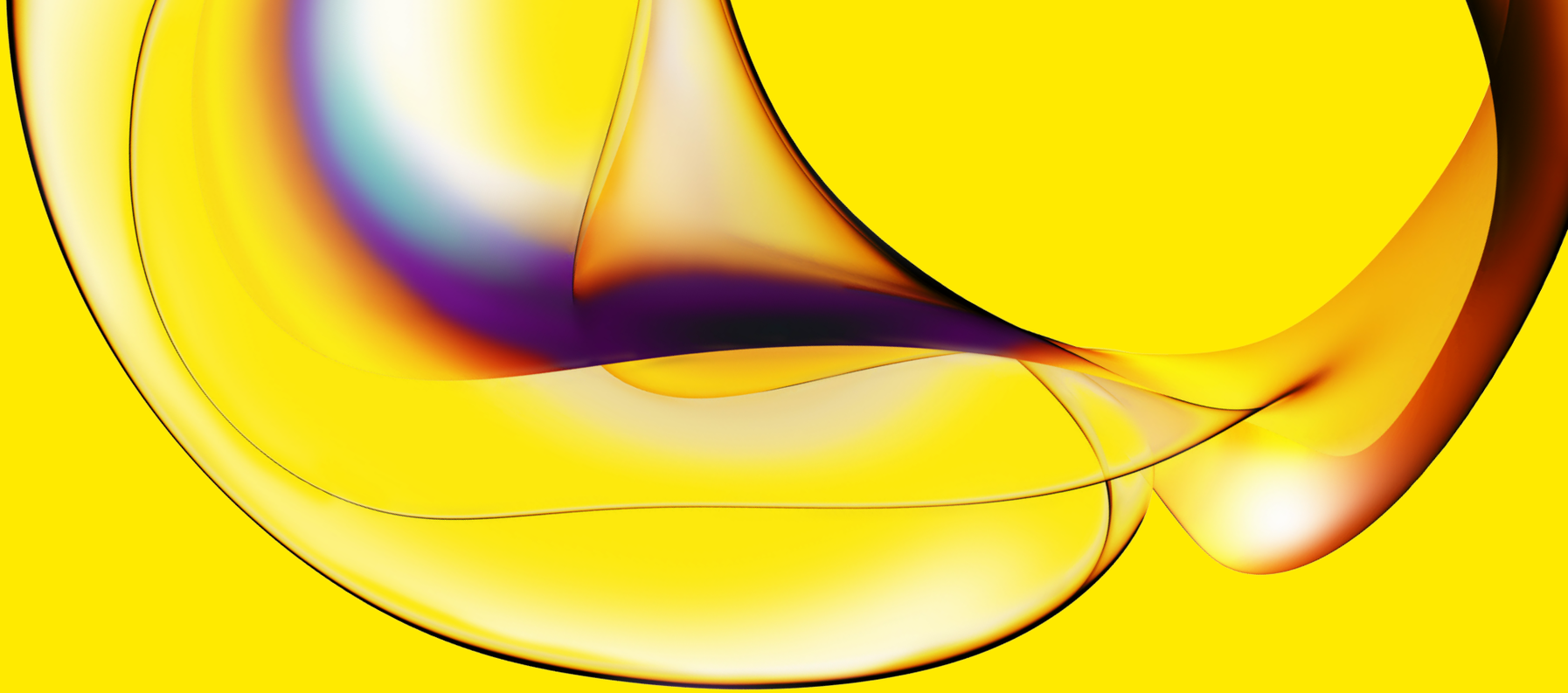

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