MANUAL

Technology: HTRF™ GPCRs

HTRF Cellular GTP / Gi protein Binding Kit

Part number	62CGTPPEG	62CGTPPEC
Test size	500 tests	20,000 tests

Storage: ≤-16°C

Version: 01 **Date:** January 2026

ASSAY PRINCIPLE

The GTP Gi cellular binding assay is intended for the simple, rapid, and direct detection of Gαi protein activation in permeabilized cells. It is an upstream readout of Gi protein coupled receptor activation. GPCR activation leads to GDP/GTP nucleotide exchange into the Gα subunit. The principle of this assay is based on HTRF™ technology. It uses a non-hydrolysable GTP analog coupled to the fluorescent Europium cryptate donor. In practice, agonist-induced GPCR stimulation leads to Gα protein conformation change, and the replacement of Gα-bound GDP by the fluorescent GTP analog in the corresponding binding pocket. Detection is made possible by the addition of d2-labeled anti-Gαi monoclonal antibody (red acceptor). When Europium cryptate and d2 are brought into close proximity, the time-resolved energy transfer between them triggers a TR-FRET signal at d2. This specific signal is proportional to the Gαi activation state. The assay enables the direct pharmacological characterization of compounds acting on Gαi-coupled receptors in in permeabilized cells. (Fig. 1).

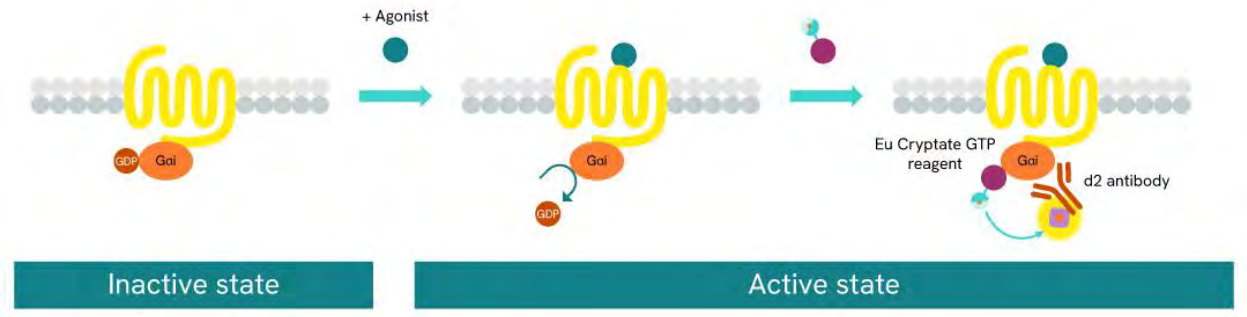
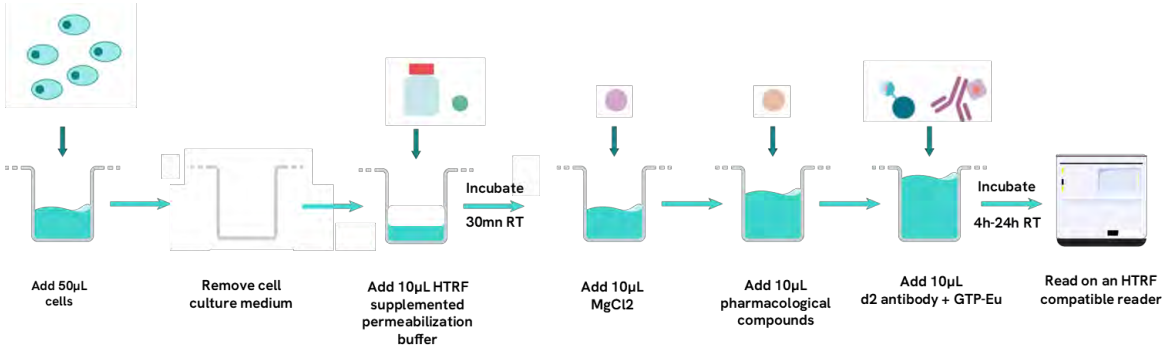


Figure 1: Principle of the HTRF GTP Gi cellular binding assay: GPCR stimulation by agonist induces GDP/GTP nucleotide exchange at the Gαi subunit, leading to the Eu-GTP analog binding to the G protein. Detection is made possible by the addition of the d2-labelled anti- Gαi antibody.

As for all HTRF assays, the calculation of the fluorescence ratio (665 nm/620 nm) removes all possible photophysical interference and means the assay is unaffected by the experimental medium conditions. This homogeneous assay is performed in a single plate, with no washing steps required.

PROTOCOL AT A GLANCE



For HTRF certified reader

For more information about HTRF™ compatible readers and for set-up recommendations, please visit our website.

MATERIAL PROVIDED

KIT COMPONENTS	STORAGE	500 TESTS			20,000 TESTS		
HTRF Cellular GTP/Gi Bind. Kit - GTP Eu	≤-16°C		red cap	2 vials - 250 µL		red cap	1 vial - 20 mL
HTRF Cellular GTP/Gi Bind. Kit - d2 Antibody	≤-16°C		blue cap	2 vials - 250 µL		green cap	1 vial - 20 mL
HTRF Permeabilization Reagent #1	≤-16°C		orange cap	1 vial - 40 µL		orange cap	1 vial – 1.3 mL
HTRF Cellular GTP/Gi Bind. Kit - MgCl2	≤-16°C		white cap	2 vials - 500 µL		white cap	1 vial - 40 mL
HTRF Cellular GTP/Gi Bind. Kit - GTP Gamma S	≤-16°C		purple cap	2 vials - 50 µL		transparent cap	1 vial - 5 mL
HTRF Cellular GTP/Gi Bind. Kit – Gi prot. Ctrl	≤-16°C		green cap	1 vial - lyophilized		green cap	2 vials - lyophilized
HTRF Stimulation Buffer #3	≤-16°C		red cap	2 vial -10 mL		red cap	4 vials – 200 mL
HTRF Permeabilization Buf. #1 (5X)	≤-16°C		yellow cap	1 vial – 1.5 mL		red cap	1 vial – 100 mL

Additional material to be used to perform the assays (not provided):

- Adherent cells
- GPCR pharmacological compounds
- CulturPlate 384-well, white, tissue-culture treated (Revvity #6007680)
- HTRF™-Certified Reader. Make sure the setup for Eu_3^+ Cryptate is used.

STORAGE AND STABILITY

Store the kit at -16°C or below until the expiration date indicated on the package.

Once reconstituted it is recommended to store Gi protein control at -70°C or below. Since the Gi protein is sensitive to degradation, keep on ice and freeze rapidely after reconstitution for storage.

Once thawed, other solutions can be frozen once.

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.

Volume of reagent aliquots should not be under 10 µL.

REAGENT PREPARATION

Before you begin

- Allow the reagents to thaw before use.
- Reagents must be prepared in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Gently centrifuging the vials after thawing, before pipetting the stock solutions is recommended.
- Working solutions must be prepared just before use.
- Prepare the working solutions from stock solutions by following the instructions below.

To prepare reagent working solutions

- **HTRF Stimulation Buffer #3**

The HTRF stimulation buffer 3 is ready to use. Thaw the buffer then use it to dilute the MgCl2, detection reagents, pharmacological compounds and Gi protein control.

- **HTRF Supplemented permeabilization buffer**

Thaw the HTRF Permeabilization buffer #1 (5X). After thawing, the HTRF Permeabilization buffer #1 (5X) may display a few aggregates which will not impair product quality. If aggregate removal is required, the HTRF Permeabilization buffer #1 (5X) can be a filtered (0.22 µm filter), prior to dilution.

Then, dilute it 5-fold in distilled water (e.g. 2.5mL of HTRF Permeabilization buffer #1 (5X) in 10mL of distilled water) to obtain the HTRF Permeabilization buffer #1 (1X).

Thaw the HTRF Permeabilization reagent #1 then dilute it 160-fold in the HTRF Permeabilization buffer #1 (1X) to obtain the HTRF supplemented permeabilization buffer.

- **MgCl2**

Thaw the MgCl2 solution. Mix gently. Stock solution is provided at 1M. MgCl2 concentration should be optimized depending on the receptor and cellular model used in the assays. Recommendations are to test the following conditions (final concentration/well): 35mM, 25mM, 15mM of MgCl2 and no MgCl2. Use the HTRF Stimulation buffer #3 to prepare the working solutions of MgCl2.

- **GTP Gamma S (negative control)**

The GTP Gamma S (GTPγS) is provided for non-specific signal determination. This non-hydrolysable GTP is used at a saturation concentration of 25 µM.

Thaw the GTPyS solution. Mix gently then dilute it 10-fold in HTRF Stimulation buffer #3 (e.g. 250µL of GTPyS solution in 2.25mL of HTRF Stimulation buffer #3) to prepare the working solution of GTPyS.

- **Gi protein control**

The Gi protein control is lyophilized. Thaw the Gi protein control at room temperature for 15 minutes. Reconstitute in HTRF Stimulation buffer #3 (supplemented or not with 35mM, 25mM, 15mM of MgCl2 depending on the conditions used for the assay) with the volume indicated on the vial to prepare the working solution of Gi protein control.

- **d2 antibody and GTP-Eu**

Thaw the d2 antibody and GTP-Eu solutions. Mix gently.

Dilute 5-fold each stock solution in HTRF Stimulation buffer #3 (e.g. 1.25mL of d2 antibody in 5mL of HTRF Stimulation buffer #3 and 1.25mL of GTP-Eu in 5mL of HTRF Stimulation buffer #3) to prepare d2 antibody and GTP-Eu stock solutions.

Mix 1 volume of d2 antibody stock solution with 1 volume of GTP-Eu stock solution (e.g. 6.25mL of d2 antibody stock solution in 6.25mL of GTP-Eu stock solution) to prepare the d2 antibody and GTP-Eu working solutions. Mix gently.

- **Pharmacological compounds (not provided)**

Pharmacological compounds must be diluted in HTRF Stimulation buffer 3 and prepared 4X.

ASSAY PROTOCOL

	Negative control	Treated cells	Non treated cells	Positive control (optional)
Step 1	Dispense 50µl/well of cells			
Step 2	Incubate for 24h at 37°C +5% CO2			
Step 3	Remove cell culture medium			
Step 4	Dispense 10µl/well of Supplemented permeabilization buffer			
Step 5	Incubate for 30mn at 20°C (+/-1°C)			
Step 6	Dispense 10µl/well of MgCl2 working solutions (concentration to be optimized: Test 35mM vs 25mM vs 15mM vs 0)			
Step 7	Dispense 10µl/well of GTPγS working solution	Dispense 10µl/well of pharmacological compounds	Dispense 10µl/well of HTRF Stimulation buffer #3	Dispense 10µl/well of Gi protein control working solution
Step 8	Dispense 10µl/well GTP Eu + Ab Gi d2 working solutions			
Step 9	Incubate for 4h at 20C° (+/-1°C)			
Step 10	Read with usual HTRF setup			
Step 11	Incubate for 24h at 20C° (+/-1°C)			
Step 12	Read with usual HTRF setup			

Please note that it is possible to run the assay as a 96-well format by dispensing 100µL of cells instead of 50µL in step 1, then 25µL of reagents or buffers instead of 10µL in steps 4, 6, 7 and 8.

CulturPlate 96-well, white, tissue-culture treated (Revvity #6055680) are recommended to run the 96-well format.

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) Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit our website.

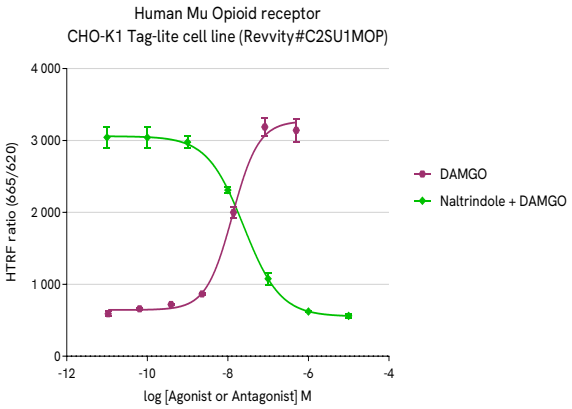
RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example (readouts on an HTRF compatible reader). Results may vary from one HTRF compatible reader to another.

Pharmacological responses in CHO-K1 Tag-lite™ Human Mu opioid receptor cell line (Revvity#C2SU1MOP): The assay was performed using the optimized conditions for this cellular model. HTRF Stimulation Buffer #3 was supplemented with 25 mM of MgCl2. The assay was performed using 80 000 cells/well. Reading was performed after 4h incubation time. Pharmacological profiles and potency values obtained with DAMGO and Naltrindole are consistent with literature.

		Agonist (DAMGO)	
		Ratio (1)	CV% (2)
Negative control		590	6%
Concentration 1	0.065 nM	659	2%
Concentration 2	0.39 nM	719	1%
Concentration 3	2.3 nM	866	1%
Concentration 4	14 nM	2001	4%
Concentration 5	83 nM	3188	4%
Concentration 6	500 nM	3143	5%

		Antagonist (Naltrindole (+ DAMGO 75nM))	
		Ratio (1)	CV% (2)
Negative control		3043	5%
Concentration 1	0.1nM	3041	5%
Concentration 2	1nM	2978	3%
Concentration 3	10nM	2310	2%
Concentration 4	100nM	1078	8%
Concentration 5	1µM	622	2%
Concentration 6	10µM	562	5%



ANALYTICAL CHARACTERISTICS

DAMGO (EC50)	13nM
Naltrindole (IC50)	24.8nM

REACH European regulations and compliance

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.

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