MANUAL

HTRF pWT BETA-ARRESTIN 2

Part # PWTBARR2

Quantity: 10 µg/10 µL H_2O

Revision: #3 of September 2023 **Store at:** -20°C

This product is intended for research purposes only. It is not intended to be used for therapeutic or diagnostic purposes.

PRODUCT DESCRIPTION

This plasmid coding for beta-arrestin 2 is intended as a companion product to the HTRF beta-arrestin 2 recruitment kit (62BDBAR2PEB/C) and to the HTRF Total Beta-arrestin 2 cellular kit (64BAR2TPEB/C). It enables the overexpression of beta-arrestin 2 in cell lines that have low endogenous levels of it, and helps to improve the resulting assay window. The use of this plasmid is especially advised in CHO cell lines that can otherwise prove difficult to use in beta-arrestin recruitment assays due to their low expression of the protein.

Plasmid name: Human beta-arrestin 2

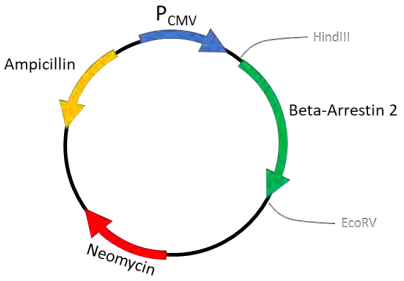
Plasmid size: 6613 bp

Beta-arrestin 2 insert size: 1229 bp

Host: Mammalian

Antibiotic resistance for E. Coli: Ampicillin

Mammalian selection marker: Neomycin



BETA-ARRESTIN 2 SEQUENCE

atgggtgaaaagcctggcaccagggtgttcaagaaaagctctccaaactgcaagctgacagtttacctggggaagagagatttcgtggaccacttggataaagtggaccctgttgatggagtggtgctg
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tgcgcctgaagggaatgaaggatgatgattatgatgatcagctttgttga

MATERIALS

REAGENTS	VOLUME CAT # PWTBARR2
pWT Beta-arrestin 2	1 vial (10µg/10µLin pure H2O)

NOT PROVIDED	CAT #
CULTURPLATE-96 +LID /50W PROXIPLATE-384 PLUS /50W	6005680 6008280
Empty plasmid	here: pcDNA 3.1
HTRF Total Beta-arrestin 2 cellular kit (1000 tests)*	64BAR2TPEB
HTRF Beta-arrestin recruitment kit (1000 tests)*	62BDBAR2PEB

*For HTRF microplate recommendations, please visit www.revvity.com

For reading, an HTRF®-compatible reader is needed. Make sure you use the appropriate setup.

For a list of HTRF®-compatible readers and setup recommendations, please visit www.revvity.com

RECOMMENDED REAGENTS:

For HEK-293 and CHO-K1 cell lines:

- Culture medium for HEK293: DMEM (Life technologies Cat.#31966021) supplemented with 10% fetal bovine serum (Eurobio CVFSVF00-01), Penicillin-Streptomycin 1X (Life technologies Cat.# 15070-063), HEPES 2mM (Life technologies Cat.#15630-122), MEM 1X(Life Technologies Cat.#11140-035), 1 mg/ml geneticin (for stable cell lines only)
- Culture medium for CHO-K1: Ham F12 (Life technologieCat.#11765054), supplemented with 10% fetal bovine serum (Eurobio CVFSVF00-01), HEPES 2mM (Life technologies Cat.#15630-122), Penicillin-Streptomycin 1X (Life technologies Cat.# 15070-063), 1 mg/ml geneticine (for stable cell lines only)
- PBS (Life technologies Cat.# 10010-031)
- Cell dissociation buffer (Sigma Cat.#C5914) for HEK-293 and Trysin-EDTA (Life technologie Cat.#25300054) for CHO cell lines.
- Opti-MEM, with GlutaMAX and phenol red (Life technologies Cat.# 51985-026)
- Lipofectamine™ 2000 (Life technologies Cat.#11668-027)

TRANSIENT TRANSFECTION MANUAL - 96 WELL PLATE

One day before the transfection, seed the cells at 80,000 cells /well in a 96 well plate (CULTURPLATE-96). For an efficient transfection, cells should be adherent with a confluency around 70 - 80%.

Our recommendation is to test 3 different quantities (5, 10, and 20 ng) of beta-arrestin 2 DNA for better mapping of the best condition for the Beta-arrestin 2 recruitment assay: optimal assay window (AW) is obtained when the Beta arrestin2 DeltaF is between 1500% and 4500%.

Example for the preparation of a mix allowing Beta arrestin 2 and AP2 detections, and a dose response with a compound detected by the Beta arrestin 2 recruitment kit for one quantity of Beta arrestin 2 DNA. A volume of 2.5 ml of mix transfection should be prepared as described in the figure below.

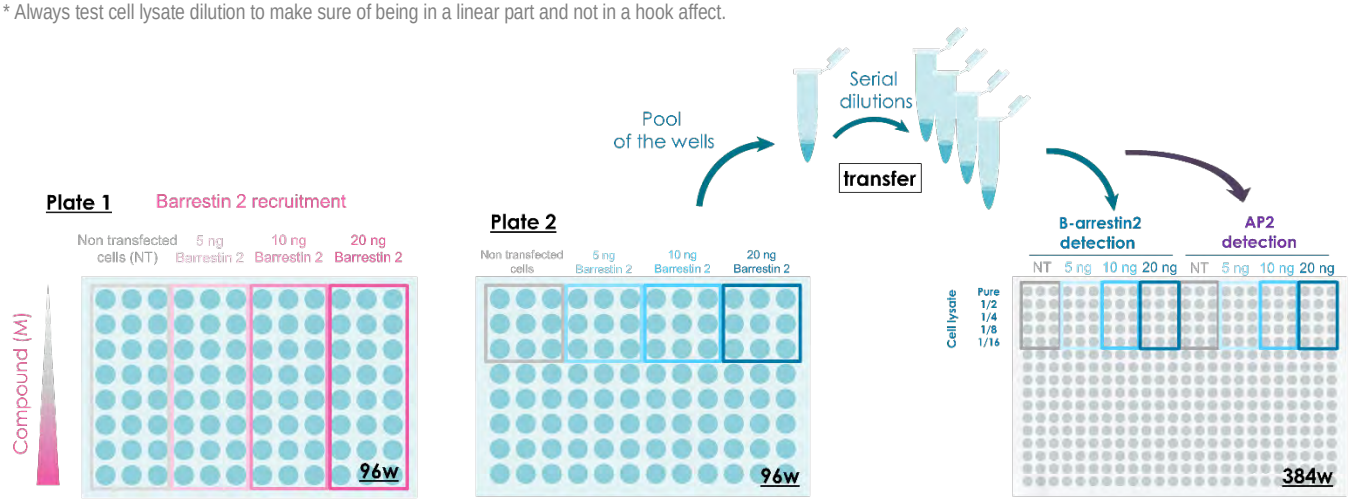
For the preparation one transfection mix (2.5 mL) per quantity of beta-arrestin 2 DNA tested, perform the following steps:

Step 1	**Lipofectamine™ preparation**	• Add 30µL of lipofectamine 2000 in 2460µL of OptiMEM • Wait for 5 minutes
Step 2	**pWT Beta-arrestin 2 plasmid dilution 1/10 (0.1 µg/µL)**	• Dilute 10µL of pWT Beta-arrestin 2 plasmid at 1µg/µL in 90 µL of ultra-pure water
Step 3	**DNA addition (150 ng total DNA)**	• Add volume of diluted pWT Beta-arrestin 2 1/10 (at 0.1 µg/µL) and pc-DNA 3.1 (at 1 µg/µL) to the lipofectamine preparation (See table below) • Wait for 20 min
Step 4	**Transfection mix addition**	• Gently remove the cell medium by aspiration • Add 50 µL of the transfection mix to the cells • Incubate for 6h at 37°C + 5% Co2, then add 100 µL of medium + SVF • Incubate 24h, then perform the 3 assays described below

Table for DNA transfection:		**Control**	**Quantity of Beta-arrestin 2**		
DNA quantities tested	pWT Beta-arrestin 2	0 ng	5 ng	10 ng	20 ng
	pcDNA 3.1	150 ng	145 ng	140 ng	130 ng
DNA volume to add for 2.5 mL de transfection mix	pWT Beta-arrestin 2 (0.1 µg/µL)	0 µL	2.5 µL	5 µL	10 µL
	pcDNA3.1 (1 µg/µL)	7.5 µL	7.3 µL	7 µL	6.5 µL

Step 5	**HTRF Beta-arrestin 2 and AP2 detections**	• Gently remove the cell medium by aspiration • Add 50 µL of Lysis buffer LB#4 • Transfer 16 µL of cell lysate (pure, 1/2, 1/4 ,1/8, and 1/16 at a minimum)*
Step 6	**HTRF Beta-arrestin 2 recruitment detection**	• Gently remove the cell medium by aspiration • Add varying concentrations of compounds • Gently remove the cell medium by aspiration • Perfom beta-arrestin 2 recruitment manual

* Always test cell lysate dilution to make sure of being in a linear part and not in a hook affect.



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.

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

3. Calculate the Delta F (ΔF).

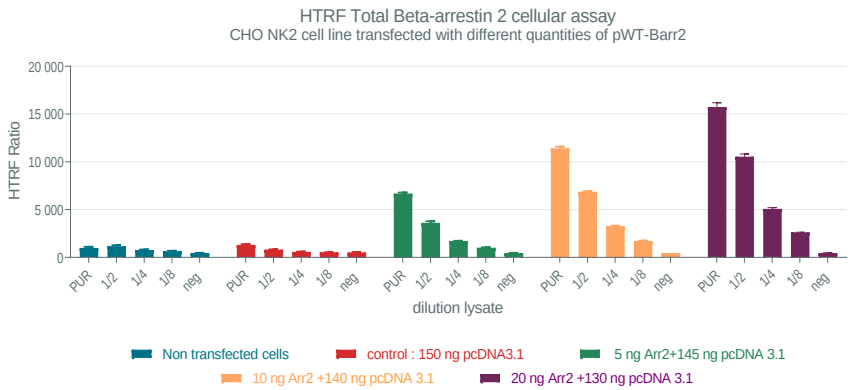
$$\Delta F = \frac{\text{Ratio}_{\text{sample}} - \text{Ratio}_{\text{background}}}{\text{Ratio}_{\text{background}}} \% = \frac{\text{Signal - Background}}{\text{Background}}$$

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

RESULTS

TYPICAL RESULTS OBTAINED USING HTRF TOTAL BETA-ARRESTIN 2 CELLULAR KIT (CAT #64BAR2TPEB) WITH THE VALISCREEN NEUROKININ NK2 (HUMAN) CHO STABLE CELL LINE (CAT# ES-251-C):

After transfection incubation time completion, the cell lysate prepared in the 96-well plate was pooled and diluted: pure, 1/2, ¼, and 1/8. Then 16 µL of lysate were transferred into a 384-well white microplate (proxiplate 384), and 4 µL of the HTRF Total Beta-arrestin 2 detection reagents were added. The HTRF signal was recorded after 3h incubation at room temperature.



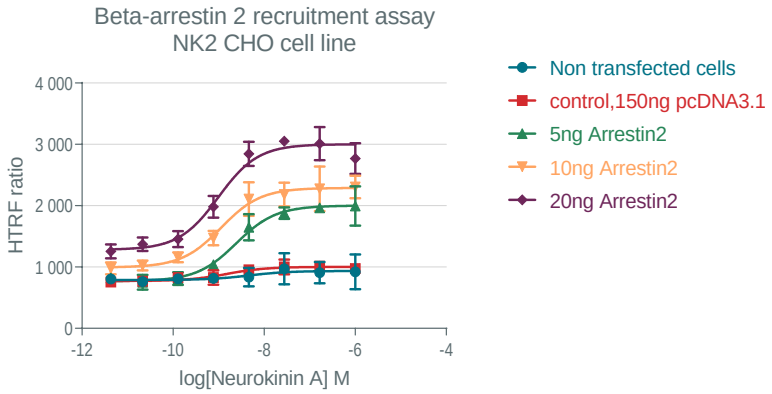
The Delta F was then calculated. Delta F is used for the comparison of day-to-day runs of the same assay or assays run by different users. It reflects the signal to background of the assay. The negative control plays the role of an internal assay control.

Delta F	Non transfected	150 ng pcDNA3.1	5 ng Arr2	10 ng Arr2	20 ng Arr2
PUR	147%	197%	1462%	2673%	3541%
1/2	192%	85%	745%	1573%	2338%

The Delta F calculated are between 1500 and 4000%, allowing a good AW (S/B) for the beta arrestin 2 recruitment assays.

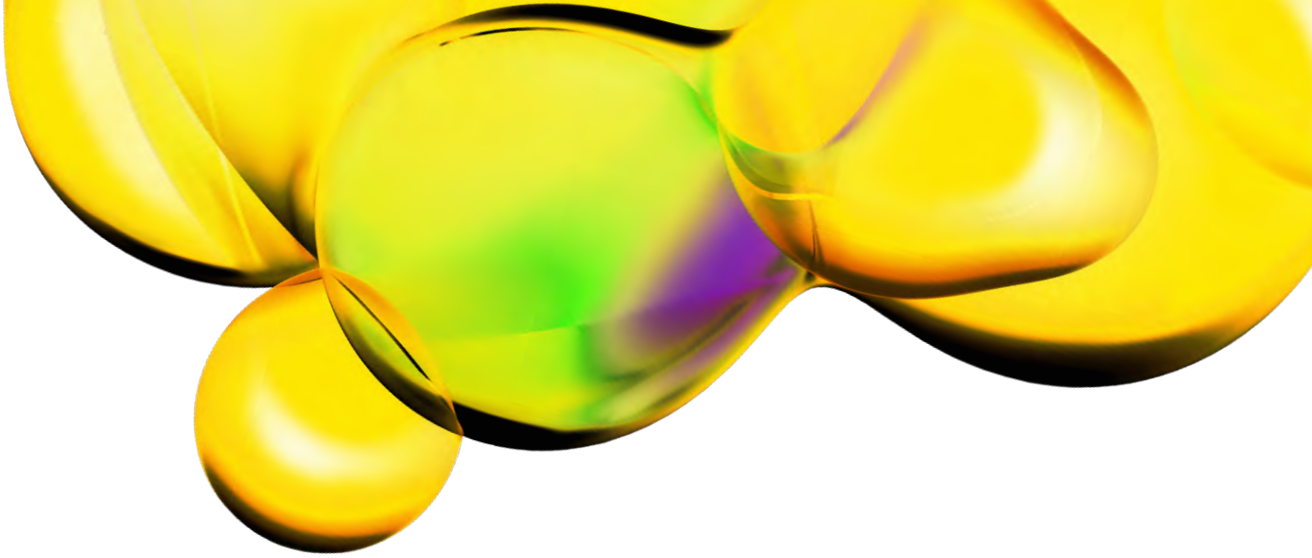
ASSOCIATED RESULTS OBTAINED WITH HTRF BETA-ARRESTIN 2 RECRUITMENT KIT (CAT #62BDBAR2PEB):

After stimulation by an agonist, the assay was performed in a parallel 96 well culture plate. The cell medium was removed from the culture plate. Cells were stabilized with 30 µL of Stabilization buffer 1 for 15 minutes at room temperature, then washed 3 times with 100µl of Wash buffer 1. Finally, 100µl of the Beta-arrestin 2 recruitment detection reagents were added. The HTRF signal was recorded after an overnight incubation at room temperature.



Delta F	Non transfected	150 ng pcDNA3.1	5 ng Arrestin2	10 ng Arrestin2	20 ng Arrestin2
S/B	1.18	1.30	2.60	2.31	2.34
Ec50 nM	4.6	1.5	2.4	1.1	1

The transfection conditions improved the S/B of the assay and increased the reliability of the assay.



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

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