revvity

APPLICATION NOTE

Highly specific tools for β–arrestin monitoring in various cells.

Key features

- Highly specific and independent detection of non-targeted β-arrestin expression level
- Ability to monitor the expression of endogenous and overexpressed β-arrestin 1 and 2
- Potential applications for a variety of cell models

Introduction

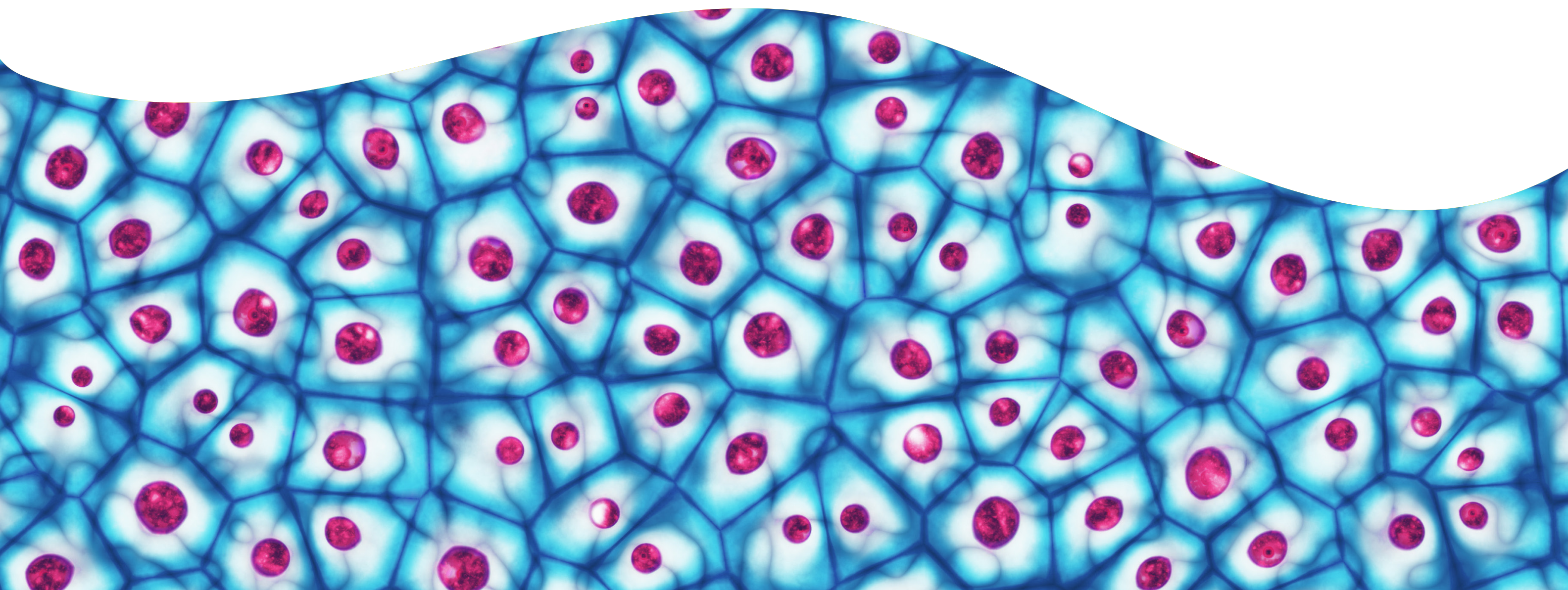
β-arrestin 1 and 2 play central roles in GPCR signaling pathways by regulating agonist-mediated GPCR signaling. Among their many roles, β-arrestins mediate both receptor desensitization and resensitization processes, act as a signaling scaffold for MAPK pathways, and target many receptors for internalization by recruiting GPCRs into adapter protein 2 complex 2 (AP-2) and clathrin-coated internalization pits. Different modes of arrestin-mediated internalization and signaling occur depending on the receptors, cell types, and expression level of β-arrestins and their partners. Furthermore, the extent of β -arrestin involvement appears to vary significantly depending on their expression, type, the receptors and ligands involved, and cell types.

The HTRF® total β-arrestin 1 (Revvity #64BAR1TPEB/C/J) and HTRF total β-arrestin 2 (Revvity #64BAR2TPEB/C/J) kits monitor the specific expression of β-arrestin 1 and 2 in cells.

Both kits are based on a TR-FRET immunoassay format. They respectively enable the detection of β-arrestin 1 and β-arrestin 2 expressed in various cellular models implicating endogenous or overexpressed arrestins.

This application note presents a convincing demonstration of the specificity of the HTRF total β-arrestin 1 and HTRF total β-arrestin 2 kits for their targeted arrestin. The data presented here also highlights the potential applications of the two kits to a variety of cellular models in studies, and observe the many differences they exhibit in terms of expression of β-arrestin 1 and β-arrestin 2.

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



Assay workflow

Principle of the HTRF Total β-arrestin 1 and 2 assays:

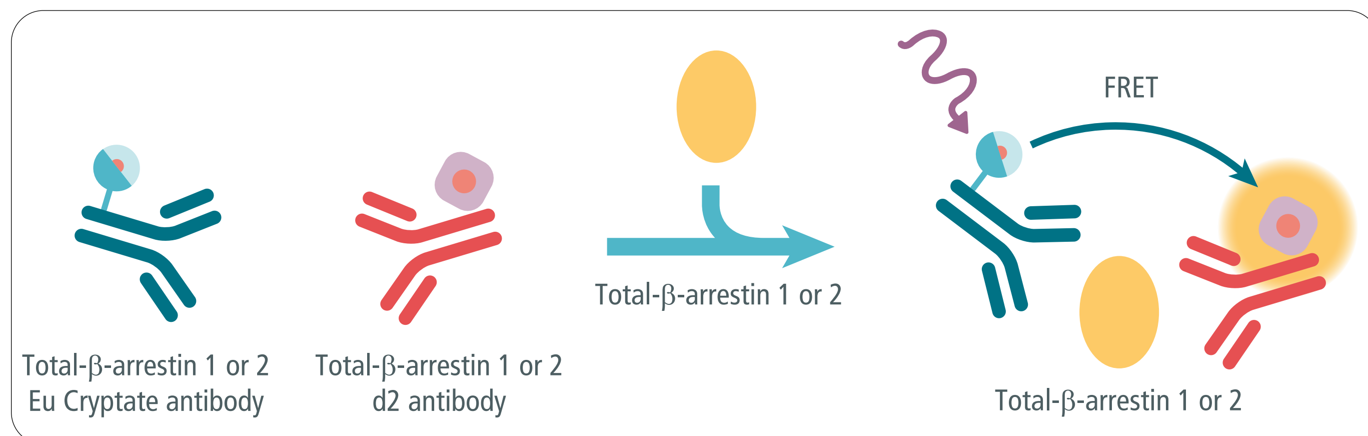


Figure 1: Principle of the HTRF Total β-arrestin 1 and 2 assays

The HTRF Total β-arrestin 1 and 2 assays can be carried out with adherent or suspension cells, with two different protocols. Both protocols are wash-free, and can either be performed in a single plate for the culture, stimulation, and lysis steps, or in two plates. In the two-plate protocol, cell lysate supernatants are transferred into the second plate for the detection step. The best detection results are generally obtained with the two-plate protocol. These HTS-designed protocols allow for assay miniaturization while maintaining robust HTRF detections.

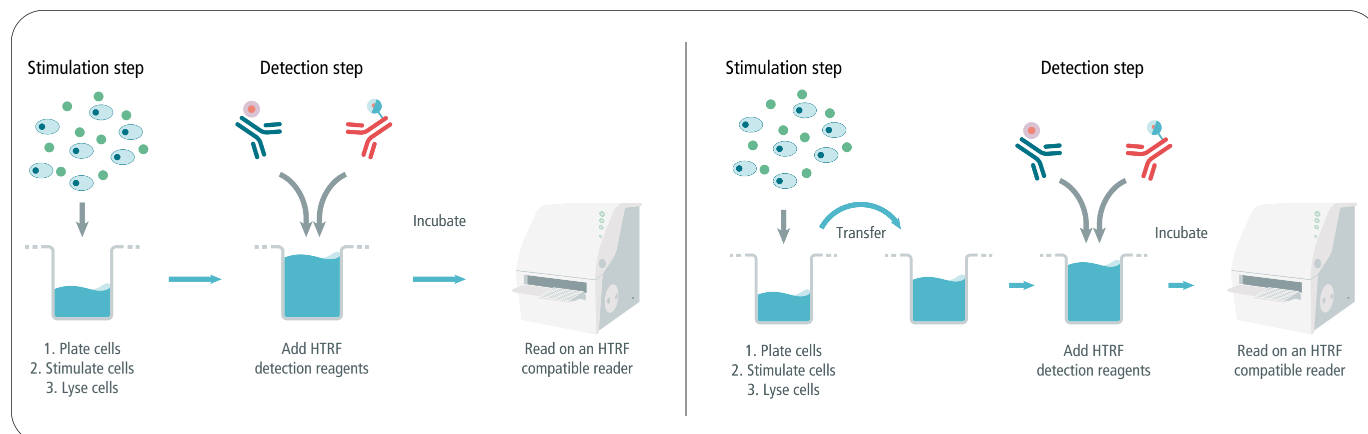


Figure 2: HTRF Total β-arrestin 1 and β-arrestin 2 assay protocols. Left panel: One-plate assay protocol. Right panel: Two-plate assay protocol.

HTRF total β-arrestin 1 and β-arrestin 2 expression assays can be performed from suspension or adherent cells cultured in 384 or 96 well plates. Following the lysis step, the expression of the β-arrestin 1 or 2 is monitored in a miniaturized format (e.g. sv 384 well plates) with HTRF reagents including pairs of anti-β-arrestin 1 or anti-β-arrestin 2 antibodies.

The antibodies are labeled with d2 (HTRF acceptor) and Europium Cryptate (HTRF donor) for both kits. Plates are incubated with the HTRF detection reagents for 3h at room temperature before reading on an HTRF compatible reader (e.g. EnVision® and VICTOR® Nivo™ multimode plate readers).

A highly specific detection of β-arrestin 1 and β-arrestin 2 in cells

The specificity of both HTRF total β-arrestin 1 kit and β-arrestin 2 kit for their respective targets was evaluated in two different cellular models:

- Double KO β-arrestin 1/2 HEK293 cells were used to demonstrate the stringent specificity of both kits.
- HEK293 cells that endogenously express both β-arrestin 1 and β-arrestin 2 were used to demonstrate that specificity in presence of both β arrestins.

Both cellular models were treated in similar experimental conditions:

- Cells were plated at 80k cell/well in a 96-w CulturPlate™ (#revvity 6005680/88/89) and incubated for 24h at 37°C and 5% CO2. Then co-transfections of various amounts (5-40ng/well) of plasmids encoding for Flag-Twin-Strep-tag®-β arrestin 1 or Flag-Twin-Strep-tag®-β-arrestin 2 were performed for 24h at 37°C and 5% CO2. Variable amounts of plasmid encoding for prK6 were also added to serve as negative controls, and were used to keep a constant final amount of DNA in all conditions.
- Cells were lysed for 30mn and cell lysate supernatants were transferred into a 384-w OptiPlate™ (#revvity 6007290/99) for the detection step with HTRF labeled antibodies from each kit.

In parallel, validations to confirm the overexpression of both arrestins were performed using the double tag detection system Flag/Twin-Strep-tag® fused to β arrestins with a pair of anti-Twin-Strep-tag®-Tb (HTRF donor) and anti-Flag-XL665 (HTRF acceptor; #revvity 61FG2XLA/B/F) as HTRF detection reagents.

The results obtained with the double tag detection system after 3h incubation at room temperature are shown in the graphs below (only one cell lysate dilution from the linear detection curve is shown here as representative data):

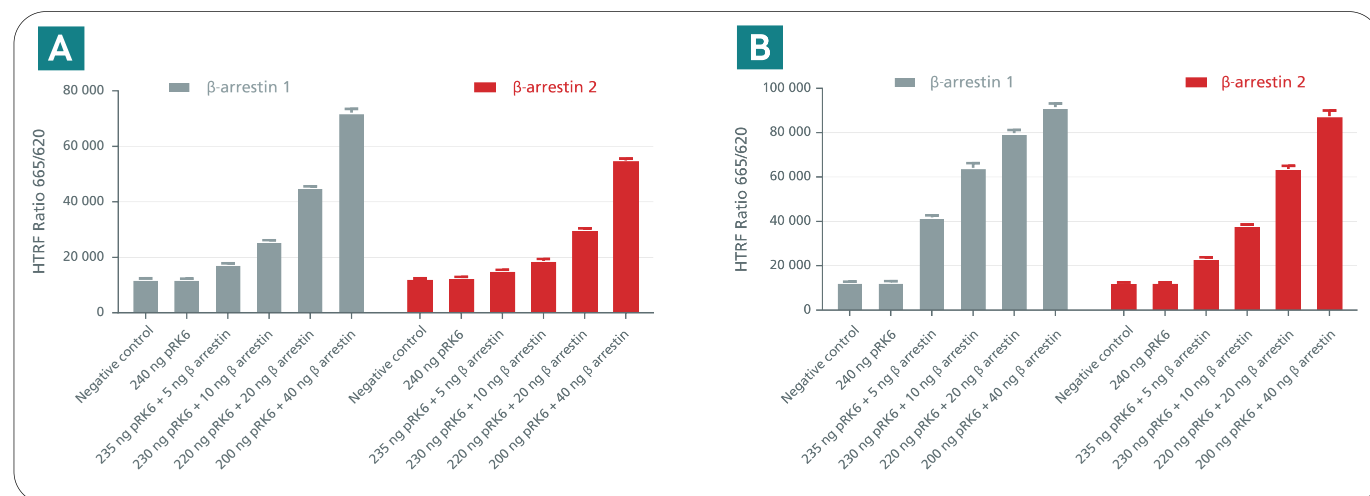


Figure 3 : Detection of Flag-Twin-Strep-tag®-β arrestin 1 and Flag-Twin-Strep-tag®-β 2 with the anti-Twin-Strep-tag®-Tb and anti-Flag-XL665 antibodies in double KO β-arrestin 1/2 HEK293 cells (A) and HEK293 cells (B).

These illustrations demonstrate the overexpression of both Flag-Twin-Strep-tag®-β arrestins transfected in the double KO β-arrestin 1/2 HEK293 and HEK293 cells, enabling accurate evaluations of the detection specificity with both HTRF total β-arrestin 1 and HTRF total β-arrestin 2 kits.

Demonstration with double KO β-arrestin 1/2 HEK 293 cells*

The specificity of the HTRF total β-arrestin 1 kit after 3h incubation at room temperature is illustrated in the figure below (only one cell lysate dilution from the linear detection curve is shown here as representative data):

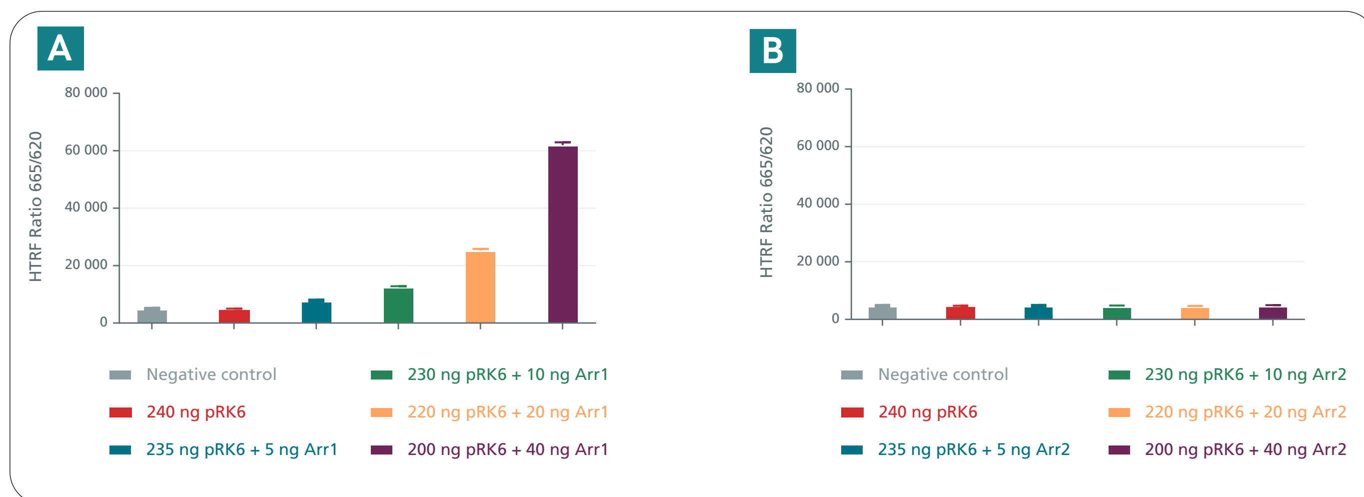


Figure 4: Detection with HTRF total β-arrestin 1 kit. Panel A: Transfections of Flag-Twin-Strep-tag®-β arrestin 1. Panel B: Transfections of Flag-Twin-Strep-tag®-β arrestin 2.

These convincingly demonstrate the highly specific detection of β-arrestin 1 with the HTRF total β- arrestin 1 kit. As shown in figure 4.B, there was no detection of β-arrestin 2, even in strongly overexpressed models.

The specificity of the HTRF total β-arrestin 2 kit after 3h incubation at room temperature is illustrated in the same way in the figure below (only one cell lysate dilution from the linear detection curve is shown here as representative data):

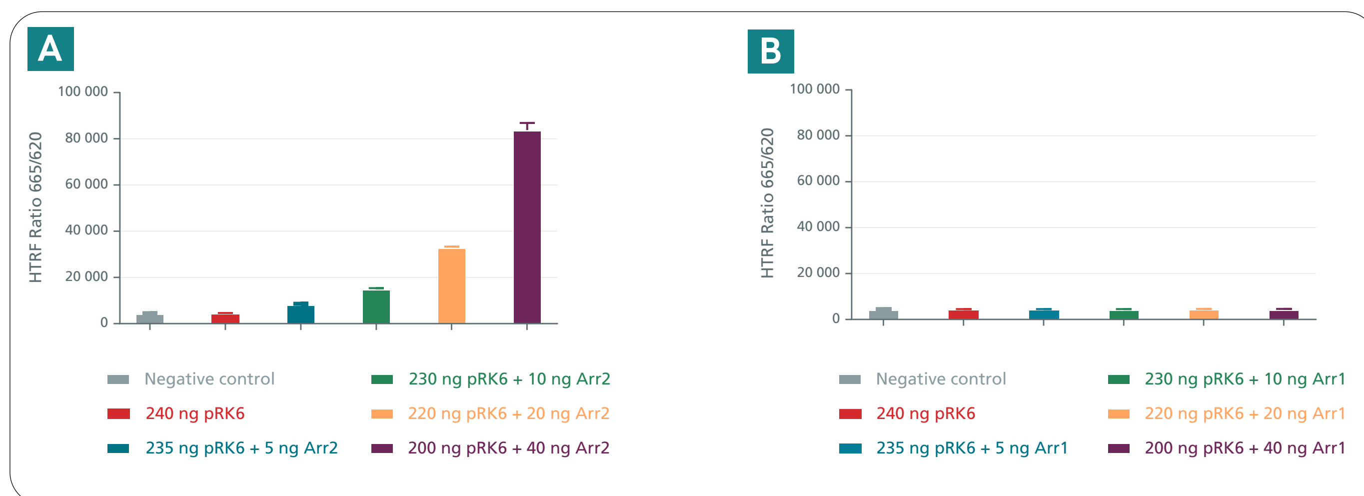


Figure 5: Detection with HTRF total β-arrestin 2 kit. Panel A: Transfections of Flag-Twin-Strep-tag®-β arrestin 2. Panel B: Transfections of Flag-Twin-Strep-tag®-β arrestin 1

These results are equally convincing and demonstrate the highly specific detection of β-arrestin 2 with the HTRF total β- arrestin 2 kit. As shown in figure 5.B, there was no detection of β-arrestin 1, even in strongly overexpressed models.

Taken together, these results demonstrate the high specificity of both HTRF total β-arrestin 1 and HTRF total β-arrestin 2 kits exclusively for their respective β-arrestin targets.

*All results obtained with the double KO β-arrestin 1/2 HEK293 cells were obtained by the Eidos team (IGF Montpellier; Drs J-P Pin & P. Rondard's team) and were kindly provided to Revvity.

Demonstration with overexpression of β-arrestin 1 and 2 in HEK 293 cells

The specificity of the HTRF total β-arrestin 1 kit after 3h incubation at room temperature is illustrated in the figure below:

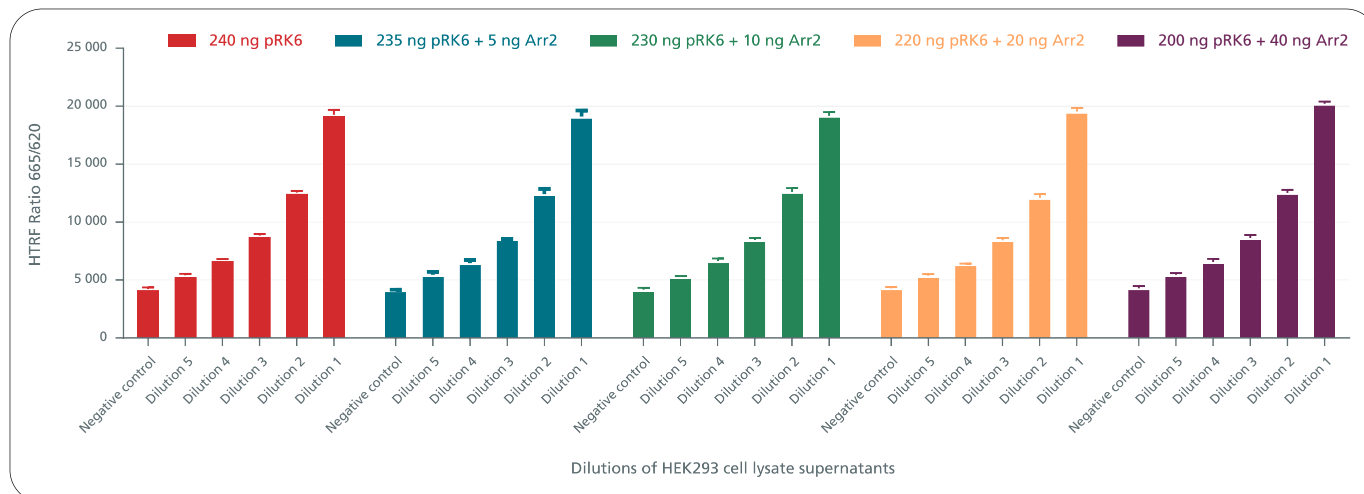


Figure 6: Measurement of β-arrestin 1 expression levels using the HTRF total β-arrestin 1 kit in HEK293 cells, with or without transfection of various amounts of Flag-Twin-Strep-tag®-β arrestin 2. Cell lysate supernatants were diluted from the highest (Dilution 1) to the lowest (Dilution 5) concentration.

These results show that the levels of β-arrestin 1 detected with the HTRF total β-arrestin 1 kit are constant whether β-arrestin 2 is absent or present in high amounts. This confirms the kit's lack of specificity for β-arrestin 2 and indicate the absence of β-arrestin 2 related interference mechanism in the expression and detection of β-arrestin 1 with the kit.

The specificity of the HTRF total β-arrestin 2 kit after 3h incubation at room temperature is illustrated in the figure below:

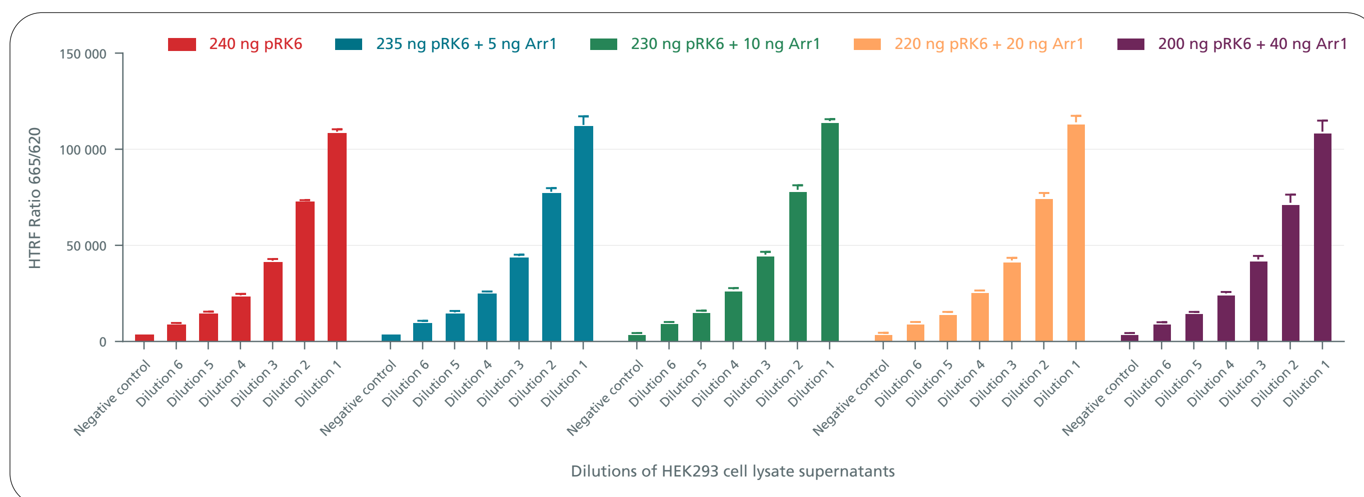


Figure 7: Measurement of Beta arrestin 2 expression level with HTRF total β-arrestin 2 kit in HEK293 cells without or with transfections of various amounts of Flag-Twin-Strep-tag®-β arrestin 1. Cell lysate supernatants were diluted from the highest (Dilution 1) to the lowest (Dilution 6) concentration.

Like the previous experiment, these results show that the levels of β-arrestin 2 detected with the HTRF total β-arrestin 2 kit are constant whether β-arrestin 1 is absent or present in high amounts. This confirms the kit's lack of specificity for β-arrestin 1 and indicate the absence of β-arrestin 1 related interference mechanism in the expression and detection of β-arrestin 2 with the kit.

Taken together, these results demonstrate the high specificity of both HTRF total β-arrestin 1 and HTRF total β-arrestin 2 kits for their respective β-arrestin targets, regardless of the expression level of the other.

Applicability of HTRF total β-arrestin 1 and 2 kits for monitoring variable endogenous expression levels of both arrestins in a panel of cellular models

To illustrate the high variability of both β-arrestin expression levels in different cells and demonstrate the applicability of the HTRF total β-arrestin 1 and 2 kits to detect them in various cells, a panel of cellular models was tested.

Cells were grown in T175 cm2 culture flasks, then lysed with lysis buffer #4 (1X) for 30 minutes at room temperature under shaking. Cell lysates from CHO-K1, Jurkat, HEK 293, NIH 3T3, MDA-MB-231, SK-OV-3, THP-1, HEP G2, TF-1, MCF7 SY-SY5Y, C2C12, HT-29, U-2-OS, HeLa, and A-431 cells were generated. For each condition, 16µL of cell lysate were transferred into a 384-well plate for the detection step. All conditions were tested in triplicate. Lysate dilutions were performed accordingly to ensure all assays were within the linear detection range of the kits.

To compare results from all cell types in a relevant way, the results presented below were normalized using the Alpha-Tubulin housekeeping cellular kit (#revvity 64ATUBPEG/H) and from only one dilution from the linear detection curve as representative data.

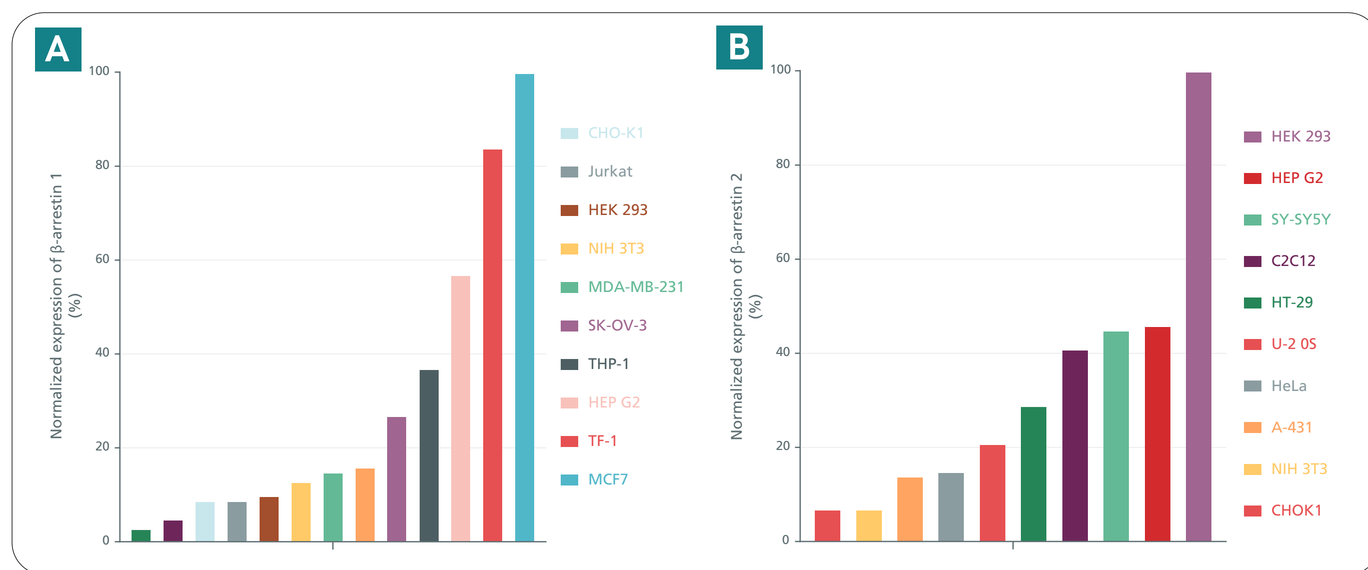


Figure 8: Measurement of β-arrestin 1 expression level with HTRF total β-arrestin 1 kit (A) and Beta arrestin 2 expression level with HTRF total β-arrestin 2 kit (B) in various cell lysates. Data were normalized with the Alpha Tubulin expression level and represented as a percentage of expression calculated with the MCF7 cells as the 100% for the β-arrestin 1 assays and HEK293 cells as the 100% for the β-arrestin 2 assays.

Taken together, these results demonstrate the high variability of endogenous expression of β-arrestin 1 and 2 in different cellular models, as well as the relevance and ability of the HTRF total β-arrestin kits to measure these differences in a large panel of cell types from different species.

Conclusion

The detection of β-arrestin 1 with the HTRF total β-arrestin 1 kit and of β-arrestin 2 with the HTRF total β-arrestin 2 kit was shown to be both highly specific and independent of the expression level of the non-targeted β-arrestin in cells. The same conclusions were reached in a HEK293 cell model transfected with siRNA β arrestin 1 and β arrestin 2 (data not shown).

The ability of the HTRF total β-arrestin kits to monitor the expression of endogenous and overexpressed β-arrestin 1 and β arrestin 2 in various cellular models was also demonstrated.

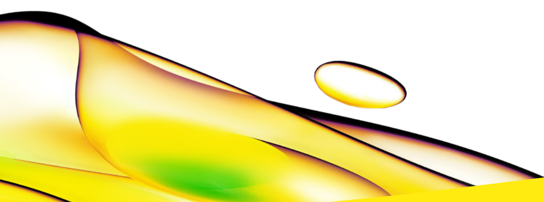
In GPCR signaling studies, monitoring the expression level of both β-arrestins and in particular β-arrestin 2 is useful to identify relevant cellular models for investigations of β-arrestin signaling pathways that rely on one β-arrestin over the other. That is the case in B-arr2 recruitment assays, which use endogenous β-arrestin 2 (B-arr2 recruitment kit #revvity 62BDBAR2PEB/C).

Author

Elodie Dupuis

Acknowledgements

We thank the Eidos team (IGF Montpellier; Drs J-P Pin & P. Rondard's team) and Arpege platform (IGF Montpellier) for their scientific contributions to this work and for the results with the double KO β-arrestin 1/2 HEK293 cells.



revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com
Copyright ©2023, Revvity, Inc. All rights reserved.