HSP90β (C-terminal Domain) TR-FRET Assay Kit

#50262
384 reactions

Description

The HSP90β (C-terminal domain) TR-FRET Assay Kit is a sensitive high-throughput screening (HTS) TR-FRET Assay Kit designed to measure the inhibition of HSP90β binding to its target protein PPDI in a homogeneous 384-well format. It utilizes a Terbium-labeled donor and a Dye-labeled acceptor to complete the FRET pairing. This TR-FRET based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The kit contains enough purified HSP90β (C-terminal Domain) Biotin Labeled, PPDI- GST-tag, labeled donor and acceptor and assay buffer for 384 reactions.

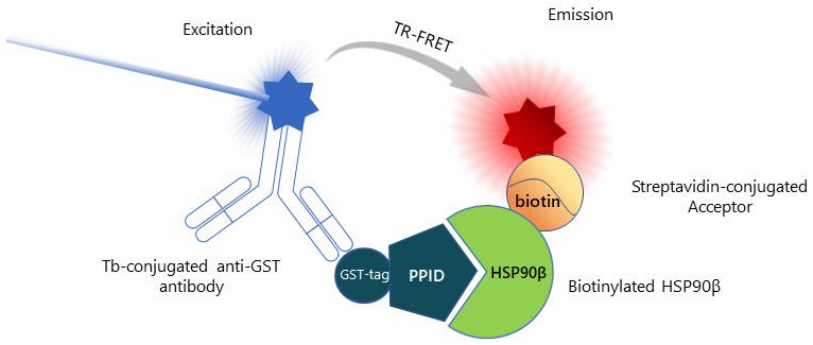

Figure 1: HSP90β (C-terminal Domain) TR-FRET Assay Kit schematic. The Terbium-labeled donor will bind to PPDI, while the Dye-labeled acceptor will bind to HSP90β. When PPID is bound to HSP90β TR-FRET occurs and it can be measured using a fluorescence plate reader.

Background

The 90 kDa Heat Shock Proteins (HSP90) are a family of molecular chaperones involved in protein folding and protein degradation. HSP90 can associate with more than 300 substrates, many of which linked to tumorigenesis. HSP90, due to its ability to stabilize proteins linked to cancer progression, has become an attractive target for cancer therapy, with several pan- HSP90 small molecule inhibitors already in clinical trials. However, targeting all HSP90 isoforms has resulted in severe complications. Contrary to the other 3 isoforms, HSP9β is cytosolic and expressed in a constitutive manner, and has more recently become the target of isoform-selective inhibitor drug development.

Application(s)

- Screening inhibitors of HSP90β binding for drug discovery and HTS applications.
- Determine compound IC_{50}.
- Perform real-time kinetic analyses.

Supplied Materials

Catalog #	Name	Amount	Storage
50313	HSP90β (C-terminal Domain) Biotin Labeled*	10 µg	-80°C
71095	PPDI- GST-Tag*	10 µg	-80°C
	Tb-labeled donor	20 µl	-20°C
	Dye-labeled acceptor	20 µl	-20°C
50324	3x HSP90 Assay Buffer 2	4 ml	-20°C
	White, nonbinding, low volume 384-well microtiter plate	1	Room Temp.

**The initial concentration of is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Fluorescence microplate reader capable of measuring Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET)
- Adjustable micropipettor and sterile tips

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. **Avoid multiple freeze/thaw cycles**.

Safety

This product is for research purposes only and not for human or therapeutic use. **The TR-FRET detection reagent contains a toxic compound**. **Use appropriate precautions.** This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

Keep final DMSO concentration at or below 1%.

Assay Protocol

All samples and controls should be tested in duplicate. We recommend pre-incubating antibodies or protein inhibitors with the target protein. For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

1. Dilute one-part **3x HSP90 Assay Buffer 2** with two-parts distilled water (3-fold dilution) to make **1x HSP90β TR-FRET Assay Buffer**. Make only enough as needed for the assay; store the remaining stock solution in aliquots at -20°C.

2. Dilute **Tb-labeled donor** and **Dye-labeled acceptor** 100-fold in 1x HSP90β TR-FRET Assay Buffer. Make only enough as needed for the assay; store the remaining stock solution in aliquots at -20°C.

3. Add 5 µl of diluted Tb-labeled donor, and 5 µl of diluted Dye-labeled acceptor to all the wells.

4. Prepare the Test Inhibitor (2 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

   4.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x HSP90β TR-FRET Assay Buffer, 10-fold more concentrated than the desired final concentrations.
   For the positive and negative controls, use 1x HSP90β TR-FRET Assay Buffer (Diluent Solution).
   OR
   4.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x HSP90β TR-FRET Assay Buffer to prepare the highest concentration of the intermediate dilutions. The concentration of DMSO is now 10%.
   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x HSP90β TR-FRET Assay Buffer. In order to keep the concentration of DMSO constant.
   For positive and negative controls, prepare 10% DMSO in 1x HSP90β TR-FRET Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

5. Add 2 µl of test inhibitor solution to each well designated “Test Inhibitor.”

6. Add 2 µl of Diluent Solution to the wells labeled “Negative Control” and “Positive Control.”

7. Thaw **HSP90β** and **PPID** on ice. Briefly spin the tube containing the protein to recover the full content of the tube. If the assay plate is going to be used more than once, prepare enough protein for this portion of the assay and aliquot the remaining protein into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

   Note: Proteins are very sensitive to freeze/thaw cycles. Do not reuse thawed aliquots or diluted protein.

8. Dilute **PPID** in 1x HSP90β TR-FRET Assay Buffer to 3 ng/µl (5 µl/well).

9. Add 5 µl of diluted PPID solution to each well labeled "Positive Control" and "Test Inhibitor."

10. Dilute **HSP90β** in 1x HSP90β TR-FRET Assay Buffer to 2 ng/µl (3 µl/ well).

11. Add 3 µl of 1x HSP90β TR-FRET Assay Buffer to the wells labeled as "Negative Control."

12. Initiate the reaction by adding 3 µl of diluted HSP90β solution to all the wells. Discard any remaining diluted HSP90β protein after use.

Component	**Negative Control**	**Positive Control**	**Test Inhibitor**
Tb-labeled donor	5 µl	5 µl	5 µl
Dye-labeled acceptor	5 µl	5 µl	5 µl
Test Inhibitor	-	-	2 µl
Diluent solution (no inhibitor)	2 µl	2 µl	-
1x HSP90β TR-FRET Buffer	5 µl	-	-
Diluted PPID	-	5 µl	5 µl
Diluted HSP90β	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

13. Incubate the plate at room temperature for 2 hours.

14. Read the TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument). The "Blank" value is subtracted from all other values.

Channel	**Variable**	**Recommended Value**
1	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	620 ± 10
	Lag time (µs)	60
	Integration time (µs)	500
2	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	665 ± 10
	Lag time (µs)	60
	Integration time (µs)	500

Calculating Results

1. Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by Dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

2. To calculate the percentage activity, the FRET value from the negative control can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\ Activity = \frac{FRET_S - FRET_{neg}}{FRET_P - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

Example Results

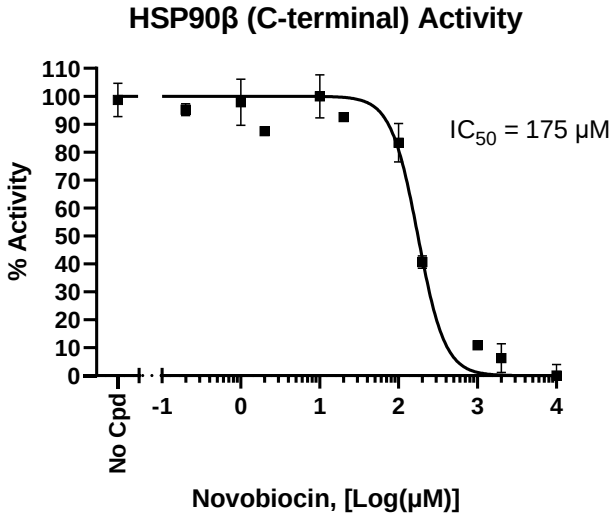

Figure 2: Inhibition of the HSP90β C-terminal Domain interaction with PPID by the inhibitor Novobiocin (BPS Bioscience #27501).
Inhibition of the interaction of HSP90β with PPID by Novobiocin was measured in the presence of increasing concentrations of Novobiocin.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

General Considerations
"Positive Control":
The "Positive Control" is the maximum signal determined upon the addition of diluent solution (for example, 1% DMSO in 1x HSP90β TR-FRET Buffer) in the absence of inhibitor.

Troubleshooting Guide
Visit. bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Reference

1. Mishra S., *et al*. The development of HSP90β-selective inhibitors to overcome detriments associated with *pan*-HPS90 inhibition. J Med Chem 64 (3), 1545-1557 (2021)
2. Khandelwal A., *et al*. Structure-guided design of an HPS90β N-terminal isoform-selective inhibitor, Nature Communications 9, 425 (2018)
3. Rahmy S., *et al*. HSP90β inhibition upregulates interferon response and enhances immune checkpoint blockade therapy in murine tumors, Front Immunol. 13, 10005045 (2022)

Related Products

Products	*Catalog #*	*Size*
HSP90α	50290	200 µg
HSP90β	50292	200 µg
HSP90β (C-terminal Domain), Biotin-labeled	50313	100 µg
PPID (CYP-40)	71095	100 µg
HSP90α (C-terminal Domain) TR-FRET Assay Kit	50261	384 reactions
HSP90α (C-terminal Domain) Inhibitor Screening Assay Kit	50317	384 reactions
HSP90β (C-terminal Domain) Inhibitor Screening Assay Kit	50314	384 reactions
HSP90α Assay Kit	50298	384 reactions
HSP90β Assay Kit	50299	384 reactions