**Description**

The HSP90α C-Terminal Domain TR-FRET Assay Kit is designed to measure binding activity of HSP90α (heat shock protein 90 kDa alpha) to its target, PPID (peptidylprolyl isomerase D) for screening and profiling applications using TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer). It utilizes Terbium-labeled donor and a dye-labeled acceptor to complete the TR-FRET pairing. The HSP90α (C-Terminal Domain) TR-FRET Assay Kit comes in a convenient 96-well format, with enough purified HSP90α (C-Terminal) amino acids 535-732), PPID, Tb-Labeled Donor and Dye-Labeled Acceptor and assay buffer for 96 reactions.

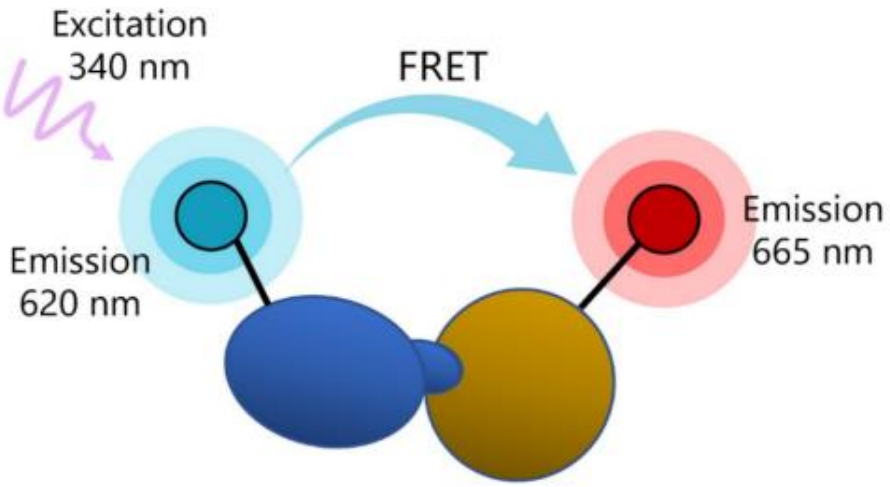


*Figure 1: Illustration of the assay principle.*

A sample containing terbium-labeled donor, dye-labeled acceptor, HSP90α (C-Terminal), PPID, and an inhibitor is incubated. The fluorescence intensity is then measured using a fluorescence reader. In the presence of binding of HSP90α (C-Terminal) to PPID, energy transfer occurs due to the proximity of the donor and acceptor. Disruption of the binding results in decrease of energy transfer. Fluorescence intensity at λ=665 nm corresponds directly to the binding of HSP90α (C-Terminal) to PPID.

**Background**

HSP90 (heat shock protein 90 kDa) is a member of the HSP family of proteins. HSP proteins are chaperone proteins, involved in aiding proteins to fold correctly and resist heat related stress, cell signaling by acting on hormone receptors and kinases, cytoskeleton organization, cell cycle and differentiation by acting on CDK4 (cyclin dependent kinase 4), Wee1 and CDK11p110, among others. HSP90 has 3 family members, which differ in their cellular localization. HSP90A is a cytosolic protein and has two inducible isoforms (HSP90α1 and α2) and one that is constitutively expressed (HSP90β). HSP90B (endoplasmin) is found in the endoplasmic reticulum, and TRAP (TNF receptor-associated protein 1) in the mitochondria. They are composed of four domains: N-terminal domain (NTD), linker, middle domain (MD) and a C-terminal domain (CTD). The NTD has a high affinity ATP binding site, while the CTD is mainly involved in target binding, dimerization and localization. HSP90α is regulated at the transcription level, being induced at high temperatures, by post-translational modifications such as phosphorylation, and by co-chaperones. HSP90 can lead to cancer progression, by participating in the stabilization of several oncogenes and proteins involved in angiogenesis, inflammation, tumor suppression and metastasis. HSP90 has thus become an attractive therapeutic target for cancer therapy. In addition to cancer, HSP90 can also play a role in Alzheimer's disease, Parkinson disease and viral infection. It is clear from its multiple and crucial functions that the development of inhibitors targeting HSP90 can prove beneficial for the treatment of several debilitating and fatal diseases.

**Applications**

- Screening inhibitors of HSP90β binding for drug discovery and high-throughput applications.
- Determine compound $IC_{50}$.
- Perform real-time kinetic analysis.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 50316 | HSP90α (C-terminal), Biotin-Labeled, His-Tag, Avi-Tag* | 5 µg | -80°C |
| 71095 | PPID (CYP-40), GST-Tag | 5 µg | -80°C |
| | Tb-Labeled Donor | 10 µl | -20°C |
| | Dye-Labeled Acceptor | 10 µl | -20°C |
| 50324 | 3x HSP90 Assay Buffer 2 | 4 ml | -20°C |
| 79685 | Black 96-well microtiter plate | 1 | Room Temp |

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

**Materials Required but Not Supplied**

- Adjustable micropipettor and sterile tips
- Fluorescence plate reader capable of measurement TR-FRET.

**Stability**

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

**Safety**

This product is for research purposes only and not for human or therapeutic use. 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**

This kit is compatible with up to 1% final DMSO concentration.

**Assay Protocol**

- All samples and controls should be performed in duplicate.
- The assay should include “Negative Control”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Novobiocin (#27501) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the $IC_{50}$ value shown in the validation data below.
- We recommend pre-incubating inhibitors with the target protein. For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

1. Prepare **1x HSP90 Assay Buffer 2** by diluting 3-fold the **3x HSP90 Assay Buffer 2** with distilled water.

   *Note: Make only the amount needed for the assay. The remaining 3x HSP90 Assay Buffer 2 can be stored as single use aliquots at -20°C.*

2. Dilute Tb-Labeled Donor 100-fold with 1x HSP90 Assay Buffer 2 (10 µl/ well).

3. Dilute Dye-Labeled Acceptor 100-fold with 1x HSP90 Assay Buffer 2 (10 µl/well).

   *Note: Make only the amount needed of diluted Tb-Labeled Donor and Dye-Labeled Acceptor for the assay. The remaining solution can be stored as single use aliquots (minimum volume of 5 µl) at -20°C.*

4. Add 10 µl of diluted Tb-Labeled Donor to all wells.

5. Add 10 µl of diluted Dye-Labeled Acceptor to all wells.

6. Prepare the Test Inhibitor (4 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 40 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x HSP90 Assay Buffer 2.

   For the positive and negative controls, use 1x HSP90 Assay Buffer 2 (Diluent Solution).

   ***OR***

   6.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x HSP90 Assay Buffer 2 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using 1x HSP90 Assay Buffer 2 containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in 1x HSP90 Assay Buffer 2 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   *Note: The final concentration of DMSO should not exceed 1%.*

7. Add 4 µl of diluted test inhibitor solution to the “Test Inhibitor” wells.

8. Add 4 µl of Diluent Solution to the “Negative Control” and “Positive Control” wells.

9. Thaw **HSP90α** and **PPID**, on ice. Briefly spin the tube to recover the full content.

10. Dilute PPID to 3 ng/µl with 1x HSP90 Assay Buffer 2 (10 µl/well).

11. Add 10 µl of diluted PPID to the "Positive Control" and "Test Inhibitor" wells.

12. Add 10 µl of 1x HSP90 Assay Buffer 2 to the "Negative Control" wells.

13. Dilute HSP90α to 2 ng/µl with 1x HSP90 Assay Buffer 2 (6 µl/well).

14. Start the reaction by adding 6 µl of diluted HSP90α to every well.

| **Component** | **Negative Control** | **Positive Control** | **Test Inhibitor** |
|---|---|---|---|
| Diluted Tb-Labeled Donor | 10 µl | 10 µl | 10 µl |
| Diluted Dye-Labeled Acceptor | 10 µl | 10 µl | 10 µl |
| Test Inhibitor | - | - | 4 µl |
| Diluent Solution | 4 µl | 4 µl | - |
| Diluted PPID (3 ng/µl) | - | 10 µl | 10 µl |
| 1x HSP90 Assay Buffer 2 | 10 µl | - | - |
| Diluted HSP90α (2 ng/µl) | 6 µl | 6 µl | 6 µl |
| **Total** | **40 µl** | **40 µl** | **40 µl** |

15. Incubate at Room Temperature for 120 minutes.

16. Read fluorescence intensity of the samples in a microplate reader capable of measuring TR-FRET.

**Instrument Settings**
Two sequential measurements should be conducted. Tb-Donor emission should be measured at 620 nm followed by Acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (620 nm emission/665 nm emission).

| Reading Mode | Time Resolved |
|---|---|
| Excitation Wavelength | 340±20 nm |
| Emission Wavelength | 620±10 nm |
| Lag Time | 60 µs |
| Integration Time | 500 µs |
| Excitation Wavelength | 340±20 nm |
| Emission Wavelength | 665±10 nm |
| Lag Time | 60 µs |
| Integration Time | 500 µs |

**Calculating Results:** Calculate the FRET value by using the following formula:

$$FRET = \frac{S_{665} - \left(\frac{Tb_{665}}{Tb_{620}} \times S_{620}\right)}{S_{620}} \times 1000$$

$S_{665}$ = Sample value measured at 665 nm, $S_{620}$ = Sample value measured at 620 nm, $Tb_{665}$ = Tb only or Blank value measured at 665 nm, $Tb_{520}$ = Tb only or Blank value measured at 520 nm.

The FRET value calculated for the negative control should be subtracted from all other measurements and can be set as 0%. The FRET value from the "Positive Control" can be set as 100% activity.

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

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{Sub}$ = FRET value for the Substrate Control, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

**Example Results**

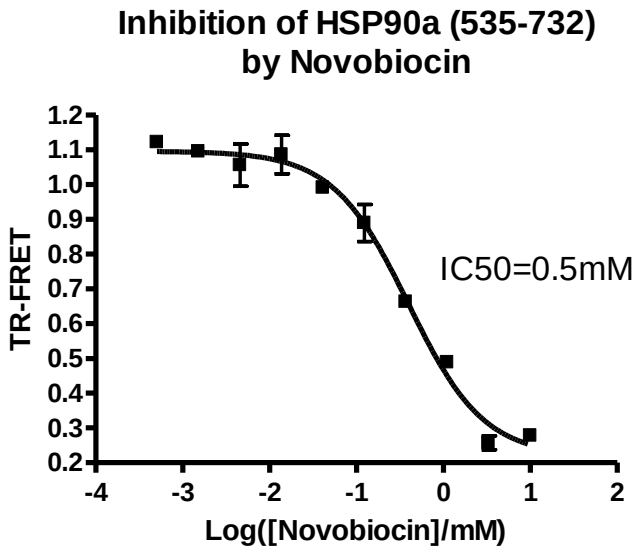


*Figure 2: Binding activity of HSP90α (C-Terminal) to PPID in the presence of Novobiocin.*
HSP90α (C-Terminal) binding to PPID was measured in the presence of increasing concentrations of Novobiocin (#27501).

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

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

**References**
Allan R.K., *et al.*, 2006 *J. Biol. Chem.* 281(11): 7161-71.
Hoter A., *et al.*, 2018 *Int J Mol Sci.* 19(9):2560.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| HSP90α (N-terminal) Assay Kit | 50298 | 384 reactions |
| HSP90β (C-Terminal Domain) TR-FRET Assay Kit | 50262 | 96 reactions/384 reactions |
| HSP90β (N-terminal) Assay Kit | 50299 | 384 reactions |
| Novobiocin | 27501 | 250 µl |
| NVP-AUY922 | 27026 | 10 mg |
| HSP90β, His-Tag Recombinant | 50292 | 200 µg |

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