Hsp90β N-Terminal Assay Kit

#50294
96 Reactions

Description

Hsp90β (heat shock protein 90) is a molecular chaperone with essential functions in maintaining transformation. Inhibition of Hsp90β function has been shown to play a role in tumorigenesis and disease progression. The Hsp90β Assay Kit is designed for the identification of Hsp90β inhibitors using fluorescence polarization. The assay is based on the competition of fluorescently labeled geldanamycin, an HSP90 inhibitor, for binding to purified recombinant Hsp90β.

The Hsp90β N-Terminal Domain Assay Kit comes in a convenient 96-well format, with enough purified Hsp90β enzyme, FITC-labeled geldanamycin, and Hsp90β assay buffer for 100 enzyme reactions. The key to the Hsp90β Assay Kit is the fluorescently labeled geldanamycin. Only one simple step on a microtiter plate is required for Hsp90β reactions. The FITC-labeled geldanamycin is incubated with a sample containing Hsp90β enzyme to produce a change in fluorescent polarization. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization.*

Applications

Study enzyme kinetics and screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage	
50292	HSP90β*	70 µg	-80°C	***Avoid multiple freeze/thaw cycles***
50312	FITC-labeled geldanamycin (2.5 µM)	30 µl	-80°C	
50311	5x Hsp90 assay buffer 1	4 ml	-20°C	
79685	Black, low binding NUNC microtiter plate	1	Room Temp	

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

Materials Required but Not Supplied

Name	Catalog #
40 M DTT	
2 mg/ml BSA (bovine serum albumin)	

Storage Conditions

This assay kit will perform optimally for up to 12 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. 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.

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Assay Protocol
Reagent Preparation

1. Thaw **FITC-labeled geldanamycin** on ice.

    a. Briefly spin tube containing FITC-labeled geldanamycin to recover full content of the tube.

    b. Aliquot into single-use aliquots. Store remaining FITC-labeled geldanamycin in aliquots at -80°C immediately.

2. Thaw **Hsp90β** on ice.

    a. Briefly spin tube containing Hsp90β to recover full content of the tube.

    b. Aliquot Hsp90β into single use aliquots. Store remaining Hsp90β in aliquots at -80°C immediately.

*Note: **FITC-labeled geldanamycin** and **Hsp90β** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

Reaction Setup
All samples and controls should be tested in duplicate.

1. Dilute FITC-labeled geldanamycin (2.5 µM stock) 25-fold with 1x Hsp90 assay buffer to make a 100 nM solution.

    Note: Make only sufficient quantity needed for the assay; store remaining 2.5 µM stock solution in aliquots at -80°C.

2. Dilute Hsp90β in 1x Hsp90 assay buffer to 35 ng/µl (700 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use.

    **Note: Optimal enzyme concentration may vary with the specific activity of the enzyme.*

3. Prepare master mix: N wells x (15 µl **5x Hsp90 assay buffer 1** + 5 µl **40 mM DTT** + 5 µl **2 mg/ml BSA** + 40 µl **H_2O**). Add 65 µl of master mixture to all wells.

Component	Blank	Enzyme Positive Control	Enzyme Negative Control	Test Inhibitor
5x Hsp90 assay buffer 1	15 µl	15 µl	15 µl	15 µl
40 mM DTT	5 µl	5 µl	5 µl	5 µl
2 mg/ml BSA	5 µl	5 µl	5 µl	5 µl
H_2O	40 µl	40 µl	40 µl	40 µl
FITC-Labeled geldanamycin (100 nM)	–	5 µl	5 µl	5 µl
Inhibitor	–	–	–	10 µl
Inhibitor Buffer (no inhibitor)	10 µl	10 µl	10 µl	–
1x HSP90 assay buffer	25 µl	–	20 µl	–
Hsp90β (35 ng/µl)	–	20 µl	–	20 µl
Total	**100 µl**	**100 µl**	**100 µl**	**100**

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4. Add 5 µl of diluted **FITC-labeled geldanamycin** (100 nM) to each well designated "Enzyme Positive Control", "Enzyme Negative Control", and "Test Inhibitor."

5. Add 10 µl of **Inhibitor** to each well designated "Test Inhibitor." For the, "Blank", "Enzyme Positive Control" and "Enzyme Negative Control", add 10 µl of the same solution without Inhibitor (**Inhibitor Buffer**).

6. Add 20 µl of **1x HSP90 assay buffer** to the well designated "Enzyme Negative Control". Add 25 µl **1x Hsp90 assay buffer** to the wells designated "Blank".

7. Initiate reaction by adding 20 µl of **diluted Hsp90β** (35 ng/µl), prepared as described above, to each well designated "Enzyme Positive Control" and "Test Inhibitor."

8. Incubate at room temperature for 2-3 hours with slow shaking.

Reaction detection and reading results
Read fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 475-495 nm and detection of emitted light ranging from 518-538 nm. Blank value is subtracted from all other values.

Calculating Results
Definition of Fluorescence Polarization:

$$P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$$

Where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}$= Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

$$mP = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right) x\ 1000$$

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000 \qquad \text{OR} \qquad mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

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Example Results

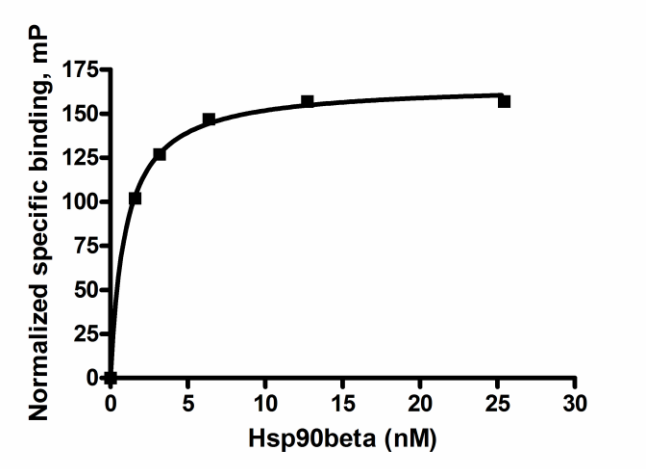

Figure 1: Binding of FITC-geldanamycin to HSP90β.
Binding was measured using the Hsp90β Assay Kit (BPS Bioscience #50294). Fluorescence was measured at λex 485nm, λem 530 nm using a Bio-Tek fluorescent microplate reader.

References

1. Kim J, et al., Biomol. Screening 2004; 9(5): 375-381.
2. Howes R, et al., Anal. Biochem. 2006; 350:202-213.

Related Products

Product	*Catalog #*	*Size*
HSP90α, His-tag Recombinant	50290	200 µg
HSP90β, His-tag Recombinant	50292	200 µg
AHA1, His-tag Recombinant	50291	200 µg
Geldanamycin	27008	5 mg
MS-275 (Entinostat)	27011	25 mg
Hsp90α N-Terminal Domain Assay Kit	50293	96 rxns
Hsp90α (N-terminal) Assay Kit	50298	384 rxns
HSP90α C-Terminal Domain TR-FRET Assay Kit	50289	96 rxns
HSP90α (C-Terminal Domain) TR-FRET Kit	50261	384 rxns
HSP90α (C-Terminal) Inhibitor Screening Assay Kit	50317	384 rxns
Hsp90β N-Terminal Domain Assay Kit	50299	384 rxns
HSP90ß (C-Terminal Domain) TR-FRET Kit	50262	384 rxns
HSP90β (C-terminal) Inhibitor Screening Kit	50314	384 rxns

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